```
* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  May 10   PROUSDDR now available on STN
 NEWS  4  May 19   PROUSDDR: One FREE connect hour, per account, in both May
                   and June 2004
 NEWS  5  May 12   EXTEND option available in structure searching
 NEWS  6  May 12   Polymer links for the POLYLINK command completed in REGISTRY
 NEWS  7  May 17   FRFULL now available on STN
 NEWS  8  May 27   New UPM (Update Code Maximum) field for more efficient patent
                   SDIs in CAplus
 NEWS  9  May 27   CAplus super roles and document types searchable in REGISTRY
 NEWS 10  May 27   Explore APOLLIT with free connect time in June 2004
 NEWS 11  Jun 22   STN Patent Forums to be held July 19-22, 2004

 NEWS EXPRESS  MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004

=> file medline
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004

 FILE LAST UPDATED: 24 JUN 2004 (20040624/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e prevelige p/au
E1            2     PREVEL MARC/AU
E2            1     PREVELAKIS V/AU
E3            0 --> PREVELIGE P/AU
```

```
E4            3      PREVELIGE P E/AU
E5           31      PREVELIGE P E JR/AU
E6            1      PREVELIGE P JR/AU
E7            1      PREVELIGE PETER E/AU
E8           10      PREVELIGE PETER E JR/AU
E9            4      PREVELIGE R/AU
E10           1      PREVEN D/AU
E11           3      PREVEN D W/AU
E12           1      PREVENDAR CRNIC ANDREJA/AU

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.76       0.97

FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 24 Jun 2004 (20040624/PD)
FILE LAST UPDATED: 24 Jun 2004 (20040624/ED)
HIGHEST GRANTED PATENT NUMBER: US6754908
HIGHEST APPLICATION PUBLICATION NUMBER: US2004123365
CA INDEXING IS CURRENT THROUGH 24 Jun 2004 (20040624/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 24 Jun 2004 (20040624/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Apr 2004
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Apr 2004

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
>>>  records and may be searched in standard search fields, e.g., /PN, <<<
>>>  /PK, etc.                                                         <<<

>>>  USPATFULL and USPAT2 can be accessed and searched together        <<<
>>>  through the new cluster USPATALL.  Type FILE USPATALL to          <<<
>>>  enter this cluster.                                               <<<
>>>                                                                    <<<
>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s us5716613/pn
L1            1 US5716613/PN

=> d l1,cbib,ab,clm

L1   ANSWER 1 OF 1  USPATFULL on STN
1998:14473 Recombinant retroviruses.
    Guber, Harry E., San Diego, CA, United States
    Jolly, Douglas J., La Jolla, CA, United States
    Respess, James G., San Diego, CA, United States
    Laikind, Paul K., San Diego, CA, United States
    Chiron Viagene, Inc., United States (U.S. corporation)
    US 5716613 19980210                                              <--
    APPLICATION: US 1995-474736  19950607 (8)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

AB Recombinant retroviruses carrying a vector construct capable of preventing, inhibiting, stabilizing or reversing infectious, cancerous or auto-immune diseases are disclosed. More specifically, the recombinant retroviruses of the present invention are useful for (a) stimulating a specific immune response to an antigen or a pathogenic antigen; (b) inhibiting a function of a pathogenic agent, such as a virus; and (c) inhibiting the interaction of an agent with a host cell receptor. In addition, eucaryotic cells infected with, and pharmaceutical compositions containing such a recombinant retrovirus are disclosed. Various methods for producing recombinant retroviruses having unique characteristics, and methods for producing transgenic packaging animals or insects are also disclosed.

CLM What is claimed is:

1. A pharmaceutical composition comprising a physiologically acceptable carrier or diluent and a replication-defective recombinant retrovirus construct which directs the expression of at least one viral or cancer antigen or mutated form thereof eliciting a cell-mediated immune response directed to said viral or cancer antigen or mutated form thereof within a human, and wherein said vital antigen is from a virus pathogenic to humans.

2. The pharmaceutical composition of claim 1 wherein the expressed antigen elicits an HLA class I-restricted immune response.

3. The pharmaceutical composition of claim 1 wherein the expressed antigen is an HIV protein or mutated form thereof.

4. The pharmaceutical composition of claim 1, wherein said cancer antigen is selected from the group consisting of a cervical carcinoma antigen, a leukemia antigen, a prostate cancer antigen, a colon cancer antigen, and a melanoma antigen.

5. The pharmaceutical composition of claim 1, wherein said cancer antigen is selected from the group consisting of an HPV antigen, an HTLV I antigen, prostate specific antigen, mutated p53 protein, and GD 2 antigen.

6. The pharmaceutical composition of claim 2 wherein the expressed antigen elicits an HLA Class II-restricted immune response.

7. The pharmaceutical composition of claim 3, wherein the HIV protein is an HIV envelope protein.

=> s us5789245/pn
L2 1 US5789245/PN

=> d l2,cbib,ab,clm

L2 ANSWER 1 OF 1 USPATFULL on STN
1998:91872 Alphavirus structural protein expression cassettes.
Dubensky, Jr., Thomas W., Rancho Sante Fe, CA, United States
Polo, John M., San Diego, CA, United States
Ibanez, Carlos E., San Diego, CA, United States
Chang, Stephen M. W., San Diego, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Driver, David A., San Diego, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5789245 19980804 <--
APPLICATION: US 1996-741881 19961030 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides compositions and methods for utilizing recombinant alphavirus vectors. Also disclosed are compositions and methods for making and utilizing eukaryotic layered vector initiation

CLM What is claimed is:

1. A DNA alphavirus structural protein expression cassette, comprising an inducible promoter and an alphavirus structural protein gene, wherein the promoter directs the expression of the alphavirus structural protein gene upon induction of the promoter within a cell, and wherein prior to induction within the cell, the expression cassette does not express sufficient quantities of structural proteins to be cytotoxic to a BHK cell containing the expression cassette.

2. A DNA alphavirus structural protein expression cassette, comprising a promoter 5' of viral cDNA which initiates the synthesis of RNA from the viral cDNA within a cell, followed by a 5' sequence which initiates transcription of alphavirus RNA from viral RNA, a viral junction region promoter operably-linked to one or more alphavirus structural protein genes and an alphavirus RNA polymerase recognition sequence, with the proviso that the cassette does not direct the expression of all alphavirus nonstructural protein genes.

3. An alphavirus structural protein expression cassette, comprising a promoter and an alphavirus glycoprotein gene, wherein the promoter directs the expression of the alphavirus glycoprotein gene, with the proviso that the promoter does not direct the expression of an alphavirus capsid protein gene.

4. An alphavirus structural protein expression cassette, comprising a promoter and an alphavirus structural protein gene, wherein the promoter directs the expression of the alphavirus structural protein gene, with the proviso that the promoter does not direct the expression of an alphavirus glycoprotein gene, nor the expression of all alphavirus nonstructural protein genes.

5. An alphavirus structural protein expression cassette, comprising a promoter, an alphavirus structural protein gene, and a heterologous ligand sequence, wherein the promoter directs the expression of the alphavirus structural protein gene and the heterologous ligand sequence, with the proviso that the promoter does not direct the expression of all alphavirus nonstructural protein genes.

6. An expression cassette according to claim 1, with the proviso that the promoter does not direct the expression of all alphavirus nonstructural protein genes.

7. An expression cassette according to claim 3, with the proviso that the promoter does not direct the expression of all alphavirus nonstructural protein genes.

8. The expression cassette according to any one of claims 1 to 7 wherein the alphavirus structural protein gene or glycoprotein gene is from a Venezuelan equine encephalitis virus.

9. The expression cassette according to any one of claims 1 to 7 wherein the alphavirus structural protein gene or glycoprotein gene is from a Ross River virus.

10. The expression cassette according to any one of claims 1 to 7 wherein the alphavirus structural protein gene or glycoprotein gene is from a Semliki Forest virus.

11. The expression cassette according to any one of claims 1 to 7 wherein the alphavirus structural protein gene or glycoprotein gene is from a Sindbis virus.

12. The expression cassette according to any one of claims 1, 2, 4, 5 or 6, wherein the alphavirus structural protein gene encodes an alphavirus capsid protein.

13. The expression cassette of any one of claims 1, 2, 3, 5, 6 or 7, wherein the alphavirus structural protein gene or glycoprotein gene encodes a protein selected from the group consisting of alphavirus structural proteins E3, E2 and E1.

14. The expression cassette according to any one of claims 3, 4, 5, or 7, wherein the cassette is a DNA cassette.

15. The expression cassette according to any one of claims 2, 3, 4, 5, 6, or 7, wherein the promoter is an inducible promoter which directs the expression of a protein within a cell upon induction of the promoter by an inducer.

16. The expression cassette according to claim 14 wherein the promoter is selected from the group consisting of metallothionein, heat shock protein 65, heat shock protein 85, and MMTV.

17. The expression cassette according to claim 14 wherein the promoter is selected from the group consisting of Drosophila actin 5C distal, SV40, Py, RSV, BK, JC, MuLV, CMV, and VA1RNA.

18. The expression cassette according to claim 3 or 7, wherein said expression cassette is an RNA expression cassette.

19. The expression cassette according to claim 4 or 5, wherein said expression cassette is an RNA expression cassette.

20. The expression cassette according to claim 18 wherein the promoter is an alphavirus junction region.

21. The expression cassette according to claim 19 wherein the promoter is an alphavirus junction region.

22. A cell containing one or more alphavirus structural protein expression cassettes according to any one of claims 1 to 7.

23. A cell according to claim 22, wherein said cell is a packaging cell, and wherein said cell, upon introduction of an alphavirus vector construct, produces recombinant alphavirus particles.

24. A method of making recombinant alphavirus particles, comprising introducing into a population of cells an alphavirus structural protein expression cassette according to any one of claims 1, 2, 5, 6 or 7, and a vector selected from the group consisting of an alphavirus vector construct, a eukaryotic layered vector initiation system, an RNA vector replicon, and a recombinant vector particle, such that recombinant alphavirus particles are produced.

25. The method according to claim 24, further comprising the step of harvesting recombinant alphavirus particles from said population of cells.

26. A method of making recombinant alphavirus particles, comprising introducing into a population of cells (a) an alphavirus structural protein expression cassette according to claim 3 or 7, (b) a vector selected from the group consisting of an alphavirus vector construct, a eukaryotic layered vector initiation system, an RNA vector replicon, and a recombinant vector particle, and (c) an expression cassette comprising a promoter and an alphavirus capsid gene, wherein the promoter directs the expression of the alphavirus capsid gene, with the proviso that the promoter does not direct the expression of an alphavirus glycoprotein gene, such that recombinant alphavirus particles are produced.

27. The method according to claim 26, further comprising the step of harvesting recombinant alphavirus particles from said population of

28. A method of making recombinant alphavirus particles, comprising introducing into a population of cells (a) an alphavirus structural protein expression cassette according to claim 18, (b) a vector selected from the group consisting of an alphavirus vector construct, a eukaryotic layered vector initiation system, an RNA vector replicon, and a recombinant vector particle, and (c) an expression cassette comprising a promoter and an alphavirus capsid gene, wherein the promoter directs the expression of the alphavirus capsid gene, with the proviso that the promoter does not direct the expression of an alphavirus glycoprotein gene, such that recombinant alphavirus particles are produced.

29. The method according to claim 28, further comprising the step of harvesting recombinant alphavirus particles from said population of cells.

```
=> file medline
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      32.52      33.49

FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004

 FILE LAST UPDATED: 24 JUN 2004 (20040624/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e prevelige p e/au
E1            2     PREVEL MARC/AU
E2            1     PREVELAKIS V/AU
E3            3 --> PREVELIGE P E/AU
E4           31     PREVELIGE P E JR/AU
E5            1     PREVELIGE P JR/AU
E6            1     PREVELIGE PETER E/AU
E7           10     PREVELIGE PETER E JR/AU
E8            4     PREVELIGE R/AU
E9            1     PREVEN D/AU
E10           3     PREVEN D W/AU
E11           1     PREVENDAR CRNIC ANDREJA/AU
E12           1     PREVENDELLO D M/AU

=> s e3 or e4 or e5
              3 "PREVELIGE P E"/AU
             31 "PREVELIGE P E JR"/AU
              1 "PREVELIGE P JR"/AU
L3           35 "PREVELIGE P E"/AU OR "PREVELIGE P E JR"/AU OR "PREVELIGE P
                JR"/AU

=> d l3,cbib,ab,1-35

L3   ANSWER 1 OF 35     MEDLINE on STN
2001643994.    PubMed ID: 11695906.   Structure of bacteriophage P22 portal
     protein in relation to assembly: investigation by Raman spectroscopy.
     Rodriguez-Casado A; Moore S D; Prevelige P E Jr; Thomas G J Jr.
```

(Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri 64110-2499, USA.) Biochemistry, (2001 Nov 13) 40 (45) 13583-91. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Salmonella phage P22, which serves as an assembly paradigm for icosahedral double-stranded DNA viruses, packages its viral genome through a capsid channel (portal) comprising 12 copies of a 725-residue subunit. Secondary and tertiary structures of the portal subunit in monomeric and dodecameric states have been investigated by Raman spectroscopy using a His6-tagged recombinant protein that self-assembles in vitro [Moore, S. D., and Prevelige, P. E., Jr. (2001) J. Biol. Chem. 276, 6779-6788]. The portal protein exhibits Raman secondary structure markers typical of a highly alpha-helical subunit fold that is little perturbed by assembly. On the other hand, Raman markers of subunit side chains change dramatically with assembly, an indication of extensive changes in side chain environments. The cysteinyl Raman signature of the portal consists of a complex pattern of sulfhydryl stretching bands, revealing diverse hydrogen-bonding states for the four S-H groups per subunit (Cys 153, Cys 173, Cys 283, and Cys 516). Upon assembly, the population of strongly hydrogen-bonded S-H groups decreases, while the population of weakly hydrogen-bonded S-H groups increases, implying that specific intrasubunit S-H.X hydrogen bonds must be weakened to effect dodecamer assembly and that the molecular mechanism involves reorganization of subunit domains without appreciable changes in domain conformations. Comparison with other viral protein assemblies suggests an assembly process not requiring metastable intermediates. The recently published X-ray structure of the phi29 portal [Simpson, A. A., et al. (2000) Nature 408, 745-750] shows that residues 125-225 lining the channel surface form alpha-helical modules spaced by short beta-strands and turns; a surprisingly close secondary structure homology is predicted for residues 240-350 of the P22 portal, despite no apparent sequence homology. This motif is proposed as an evolutionarily conserved domain involved in DNA translocation.

L3 ANSWER 2 OF 35 MEDLINE on STN

2001420295. PubMed ID: 11467958. Kinetic and calorimetric evidence for two distinct scaffolding protein binding populations within the bacteriophage P22 procapsid. Parker M H; Brouillette C G; **Prevelige P E Jr.** (Laboratory for Biological Calorimetry, Biomolecular Analysis Group, Center for Biophysical Science and Engineering, and Department of Microbiology, University of Alabama at Birmingham, 35294, USA.) Biochemistry, (2001 Jul 31) 40 (30) 8962-70. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB A wide variety of viruses require the transient presence of scaffolding proteins to direct capsid assembly. In the case of bacteriophage P22, a model in which the scaffolding protein selectively stabilizes on-pathway growing intermediates has been proposed. The stoichiometry and thermodynamics of binding of the bacteriophage P22 scaffolding protein within the procapsid were analyzed by light scattering and isothermal titration calorimetry. Calorimetric experiments carried out between 10 and 37 degrees C were consistent with the presence of at least two distinct populations of binding sites, in agreement with kinetic evidence obtained by a light scattering assay. Binding to the high-affinity sites occurred at 20 degrees C with a stoichiometry of approximately 60 scaffolding molecules per procapsid and an apparent K(d) of approximately 100-300 nM and was almost completely enthalpy-driven. For the second binding population, precise fitting of the data was impossible due to small heats of binding, but the thermodynamics of binding were clearly distinct from the high-affinity phase. The heat capacity change (DeltaC(p)()) of binding was large for the high-affinity sites and negative for both sets of sites. Addition of sodium chloride (1 M) greatly reduced the magnitude of the apparent DeltaH, in agreement with previous evidence that electrostatic interactions play a major role in binding. A mutant scaffolding protein that forms covalent dimers (R74C/L177I) bound only to the high-affinity sites. These data comprise the first quantitative measurements of the energetics of the coat protein/scaffolding protein interaction.

L3 ANSWER 3 OF 35 MEDLINE on STN

2001196526. PubMed ID: 11092883. Structural transformations accompanying the assembly of bacteriophage P22 portal protein rings in vitro. Moore S D; **Prevelige P E Jr**. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA.) Journal of biological chemistry, (2001 Mar 2) 276 (9) 6779-88. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The Salmonella typhimurium bacteriophage P22 assembles an icosahedral capsid precursor called a procapsid. The oligomeric portal protein ring, located at one vertex, comprises the conduit for DNA entry and exit. In conjunction with the DNA packaging enzymes, the portal ring is an integral component of a nanoscale machine that pumps DNA into the phage head. Although the portal vertex is assembled with high fidelity, the mechanism by which a single portal complex is incorporated during procapsid assembly remains unknown. The assembly of bacteriophage P22 portal rings has been characterized in vitro using a recombinant, His-tagged protein. Although the portal protein remained primarily unassembled within the cell, once purified, the highly soluble monomer assembled into rings at room temperature at high concentrations with a half time of approximately 1 h. Circular dichroic analysis of the monomers and rings indicated that the protein gained alpha-helicity upon polymerization. Thermal denaturation studies suggested that the rings contained an ordered domain that was not present in the unassembled monomer. A combination of 4,4'-dianilino-1,1'-binapthyl-5,5'-disulfonic acid (bis-ANS) binding fluorescence studies and limited proteolysis revealed that the N-terminal portion of the unassembled subunit is meta-stable and is susceptible to structural perturbation by bis-ANS. In conjunction with previously obtained data on the behavior of the P22 portal protein, we propose an assembly model for P22 portal rings that involves a meta-stable monomeric subunit.

L3 ANSWER 4 OF 35 MEDLINE on STN

2001195400. PubMed ID: 11178899. Hydrogen-deuterium exchange as a probe of folding and assembly in viral capsids. Tuma R; Coward L U; Kirk M C; Barnes S; **Prevelige P E Jr**. (Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL, 35294-2170, USA.) Journal of molecular biology, (2001 Feb 23) 306 (3) 389-96. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB The dynamics of proteins within large cellular assemblies are important in the molecular transformations that are required for macromolecular synthesis, transport, and metabolism. The capsid expansion (maturation) accompanying DNA packaging in the dsDNA bacteriophage P22 represents an experimentally accessible case of such a transformation. A novel method, based on hydrogen-deuterium exchange was devised to investigate the dynamics of capsid expansion. Mass spectrometric detection of deuterium incorporation allows for a sensitive and quantitative determination of hydrogen-deuterium exchange dynamics irrespective of the size of the assembly. Partial digestion of the exchanged protein with pepsin allows for region-specific assignment of the exchange. Procapsids and mature capsids were probed under native and slightly denaturing conditions. These experiments revealed regions that exhibit different degrees of flexibility in the procapsid and in the mature capsid. In addition, exchange and deuterium trapping during the process of expansion itself was observed and allowed for the identification of segments of the protein subunit that become buried or stabilized as a result of expansion. This approach may help to identify residues participating in macromolecular transformations and uncover novel patterns and hierarchies of interactions that determine functional movements within molecular machines.

L3 ANSWER 5 OF 35 MEDLINE on STN

2001142272. PubMed ID: 11170383. Characterization of subunit structural changes accompanying assembly of the bacteriophage P22 procapsid. Tuma R; Tsuruta H; Benevides J M; **Prevelige P E Jr**; Thomas G J Jr. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35205, USA.) Biochemistry, (2001 Jan 23) 40 (3) 665-74. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB ... serves as a model for the assembly and maturation of icosahedral double-stranded DNA viruses. The viral capsid precursor, or procapsid, is assembled from 420 copies of a 47 kDa coat protein subunit (gp5) that is rich in beta-strand secondary structure. Maturation to the capsid, which occurs in vivo upon DNA packaging, is accompanied by shell expansion and a large increase in the level of protection against deuterium exchange of amide NH groups. Accordingly, shell maturation resembles the final step in protein folding, wherein domain packing and an exchange-protected core become more fully developed [Tuma, R., Prevelige, P. E., Jr., and Thomas, G. J., Jr. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 9885-9890]. Here, we exploit recent advances in Raman spectroscopy to investigate the P22 coat protein subunit under conditions which stabilize the monomeric state, viz., in solution at very low concentrations. Under these conditions, the monomer exhibits an elongated shape, as demonstrated by small-angle X-ray scattering. Raman spectra allow the identification of conformation-sensitive marker bands of the monomer, as well as the characterization of NH exchange dynamics for comparison with procapsid and capsid shell assemblies. We show that procapsid assembly involves significant ordering of the predominantly beta-strand backbone. We propose that such ordering may mediate formation of the distinct subunit conformations required for assembly of a T = 7 icosahedral lattice. However, the monomer, like the subunit within the procapsid lattice, exhibits a moderate level of protection against low-temperature NH exchange, indicative of a nascent folding core. The environments and exchange characteristics of key side chains are also similar for the monomeric and procapsid subunits, and distinct from corresponding characteristics of the capsid subunit. The monomer thus represents a compact but metastable folding intermediate along the pathway to assembly of the procapsid and capsid.

L3 ANSWER 6 OF 35 MEDLINE on STN

2000229882. PubMed ID: 10764583. Structure of the coat protein-binding domain of the scaffolding protein from a double-stranded DNA virus. Sun Y; Parker M H; Weigele P; Casjens S; **Prevelige P E Jr**; Krishna N R. (Comprehensive Cancer Center, Birmingham, AL, 35294, USA.) Journal of molecular biology, (2000 Apr 14) 297 (5) 1195-202. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Scaffolding proteins are required for high fidelity assembly of most high T number dsDNA viruses such as the large bacteriophages, and the herpesvirus family. They function by transiently binding and positioning the coat protein subunits during capsid assembly. In both bacteriophage P22 and the herpesviruses the extreme scaffold C terminus is highly charged, is predicted to be an amphipathic alpha-helix, and is sufficient to bind the coat protein, suggesting a common mode of action. NMR studies show that the coat protein-binding domain of P22 scaffolding protein exhibits a helix-loop-helix motif stabilized by a hydrophobic core. One face of the motif is characterized by a high density of positive charges that could interact with the coat protein through electrostatic interactions. Results from previous studies with a truncation fragment and the observed salt sensitivity of the assembly process are explained by the NMR structure.

L3 ANSWER 7 OF 35 MEDLINE on STN

2000198313. PubMed ID: 10731416. Visualization of the maturation transition in bacteriophage P22 by electron cryomicroscopy. Zhang Z; Greene B; Thuman-Commike P A; Jakana J; **Prevelige P E Jr**; King J; Chiu W. (Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA.) Journal of molecular biology, (2000 Mar 31) 297 (3) 615-26. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Large-scale conformational transitions are involved in the life-cycle of many types of virus. The dsDNA phages, herpesviruses, and adenoviruses must undergo a maturation transition in the course of DNA packaging to convert a scaffolding-containing precursor capsid to the DNA-containing

mature virion. This conformational transition converts the procapsid, which is smaller, rounder, and displays a distinctive skewing of the hexameric capsomeres, to the mature virion, which is larger and more angular, with regular hexons. We have used electron cryomicroscopy and image reconstruction to obtain 15 A structures of both bacteriophage P22 procapsids and mature phage. The maturation transition from the procapsid to the phage results in several changes in both the conformations of the individual coat protein subunits and the interactions between neighboring subunits. The most extensive conformational transformation among these is the outward movement of the trimer clusters present at all strict and local 3-fold axes on the procapsid inner surface. As the trimer tips are the sites of scaffolding binding, this helps to explain the role of scaffolding protein in regulating assembly and maturation. We also observe DNA within the capsid packed in a manner consistent with the spool model. These structures allow us to suggest how the binding interactions of scaffolding and DNA with the coat shell may act to control the packaging of the DNA into the expanding procapsids.

L3 ANSWER 8 OF 35 MEDLINE on STN
2000193825. PubMed ID: 10729161. Identification of additional coat-scaffolding interactions in a bacteriophage P22 mutant defective in maturation. Thuman-Commike P A; Greene B; Jakana J; McGough A; **Prevelige P E**; Chiu W. (Verna and Marrs McLean, Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.) Journal of virology, (2000 Apr) 74 (8) 3871-3. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Scaffolding proteins play a critical role in the assembly of certain viruses by directing the formation and maturation of a precursor capsid. Using electron cryomicroscopy difference mapping, we have identified an altered arrangement of a mutant scaffolding within the bacteriophage P22 procapsid. This mutant scaffolding allows us to directly visualize scaffolding density within the P22 procapsid. Based on these observations we propose a model for why the mutant prevents scaffolding release and capsid maturation.

L3 ANSWER 9 OF 35 MEDLINE on STN
2000014556. PubMed ID: 10545185. Identification and characterization of the domain structure of bacteriophage P22 coat protein. Lanman J; Tuma R; **Prevelige P E Jr**. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35205, USA.) Biochemistry, (1999 Nov 2) 38 (44) 14614-23. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB The bacteriophage P22 serves as a model for assembly of icosahedral dsDNA viruses. The P22 procapsid, which constitutes the precursor for DNA packaging, is built from 420 copies of a single coat protein with the aid of stoichiometric amounts of scaffolding protein. Upon DNA entry, the procapsid shell expands and matures into a stable virion. It was proposed that expansion is mediated by hinge bending and domain movement. We have used limited proteolysis to map the dynamic stability of the coat protein domain structures. The coat protein monomer is susceptible to proteolytic digestion, but limited proteolysis by small quantities of elastase or chymotrypsin yielded two metastable fragments (domains). The N-terminal domain (residues 1-180) is linked to the C-terminal domain (residues 205-429) by a protease-susceptible loop (residues 180-205). The two domains remain associated after the loop cleavage. Although only a small change of secondary structure results from the loop cleavage, both tertiary interdomain contacts and subunit thermostability are diminished. The intact loop is also required for assembly of the monomeric coat protein into procapsids. Upon assembly, coat protein becomes largely protease-resistant, baring cleavage within the loop region of about half of the subunits. Loop cleavage decreases the stability of the procapsids and facilitates heat-induced shell expansion. Upon expansion, the loop becomes protease-resistant. Our data suggest the loop region becomes more ordered during assembly and maturation and thereby plays an important role in both of these stages.

L3 ANSWER 10 OF 35 MEDLINE on STN
1999284851. PubMed ID: 10354452. Mechanism of scaffolding-directed virus assembly suggested by comparison of scaffolding-containing and scaffolding-lacking P22 procapsids. Thuman-Commike P A; Greene B; Malinski J A; Burbea M; McGough A; Chiu W; **Prevelige P E Jr**. (Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.. pthuman@bcm.tmc.edu) . Biophysical journal, (1999 Jun) 76 (6) 3267-77. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB Assembly of certain classes of bacterial and animal viruses requires the transient presence of molecules known as scaffolding proteins, which are essential for the assembly of the precursor procapsid. To assemble a procapsid of the proper size, each viral coat subunit must adopt the correct quasiequivalent conformation from several possible choices, depending upon the T number of the capsid. In the absence of scaffolding protein, the viral coat proteins form aberrantly shaped and incorrectly sized capsids that cannot package DNA. Although scaffolding proteins do not form icosahedral cores within procapsids, an icosahedrally ordered coat/scaffolding interaction could explain how scaffolding can cause conformational differences between coat subunits. To identify the interaction sites of scaffolding protein with the bacteriophage P22 coat protein lattice, we have determined electron cryomicroscopy structures of scaffolding-containing and scaffolding-lacking procapsids. The resulting difference maps suggest specific interactions of scaffolding protein with only four of the seven quasiequivalent coat protein conformations in the T = 7 P22 procapsid lattice, supporting the idea that the conformational switching of a coat subunit is regulated by the type of interactions it undergoes with the scaffolding protein. Based on these results, we propose a model for P22 procapsid assembly that involves alternating steps in which first coat, then scaffolding subunits form self-interactions that promote the addition of the other protein. Together, the coat and scaffolding provide overlapping sets of binding interactions that drive the formation of the procapsid.

L3 ANSWER 11 OF 35 MEDLINE on STN
1999196778. PubMed ID: 10096920. Solution x-ray scattering-based estimation of electron cryomicroscopy imaging parameters for reconstruction of virus particles. Thuman-Commike P A; Tsuruta H; Greene B; **Prevelige P E Jr**; King J; Chiu W. (Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030, USA.) Biophysical journal, (1999 Apr) 76 (4) 2249-61. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB Structure factor amplitudes and phases can be computed directly from electron cryomicroscopy images. Inherent aberrations of the electromagnetic lenses and other instrumental factors affect the structure factors, however, resulting in decreased accuracy in the determined three-dimensional reconstruction. In contrast, solution x-ray scattering provides absolute and accurate measurement of spherically averaged structure factor amplitudes of particles in solution but does not provide information on the phases. In the present study, we explore the merits of using solution x-ray scattering data to estimate the imaging parameters necessary to make corrections to the structure factor amplitudes derived from electron cryomicroscopic images of icosahedral virus particles. Using 400-kV spot-scan images of the bacteriophage P22 procapsid, we have calculated an amplitude contrast of 8.0 +/- 5.2%. The amplitude decay parameter has been estimated to be 523 +/- 188 A2 with image noise compensation and 44 +/- 66 A2 without it. These results can also be used to estimate the minimum number of virus particles needed for reconstruction at different resolutions.

L3 ANSWER 12 OF 35 MEDLINE on STN
1999194856. PubMed ID: 10092457. Cavity defects in the procapsid of bacteriophage P22 and the mechanism of capsid maturation. de Sousa P C Jr; Tuma R; **Prevelige P E Jr**; Silva J L; Foguel D. (Programa de Biologia Estrutural, Departamento de Bioquimica Medica - ICB Centro Nacional de

Rio de Janeiro, Rio de Janeiro, RJ, 21941-590, Brazil.) Journal of molecular biology, (1999 Apr 2) 287 (3) 527-38. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Bacteriophage P22 belongs to a family of double-stranded DNA viruses that share common morphogenetic features like DNA packaging into a procapsid precursor and maturation. Maturation involves cooperative expansion of the procapsid shell with concomitant lattice stabilization. The expansion is thought to be mediated by movement of two coat protein domains around a hinge. The metastable conformation of subunit within the procapsid lattice is considered to constitute a late folding intermediate. In order to understand the mechanism of expansion it is necessary to characterize the interactions stabilizing procapsid and mature capsid lattices, respectively. We employ pressure dissociation to compare subunit packing within the procapsid and expanded lattice. Procapsid shells contain larger cavities than the expanded shells, presumably due to polypeptide packing defects. These defects contribute to the metastable nature of the procapsid lattice and are cured during expansion. Improved packing contributes to the increased stability of the expanded shell. Comparison of two temperature-sensitive folding (tsf) mutants of coat protein (T294I and W48Q) with wild-type coat revealed that both mutations markedly destabilized the procapsid shell and yet had little effect on relative stability of the monomeric subunit. Thus, the regions affected by these packing defects constitute subunit interfaces of the procapsid shell. The larger activation volume of pressure dissociation observed for both T294I and W48Q indicates that the decreased stability of these particles is due to increase of cavity defects. These defects in the procapsid lattice are cured upon expansion suggesting that the intersubunit contacts affected by tsf mutations are absent or rearranged in the mature shell. The energetics of the in vitro expansion reaction also suggests that entropic stabilization contributes to the large free energy barrier for expansion. Copyright 1999 Academic Press.

L3 ANSWER 13 OF 35 MEDLINE on STN

1999045444. PubMed ID: 9826587. Local rules simulation of the kinetics of virus capsid self-assembly. Schwartz R; Shor P W; **Prevelige P E Jr;** Berger B. (Laboratory for Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.) Biophysical journal, (1998 Dec) 75 (6) 2626-36. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB A computer model is described for studying the kinetics of the self-assembly of icosahedral viral capsids. Solution of this problem is crucial to an understanding of the viral life cycle, which currently cannot be adequately addressed through laboratory techniques. The abstract simulation model employed to address this is based on the local rules theory of. Proc. Natl. Acad. Sci. USA. 91:7732-7736). It is shown that the principle of local rules, generalized with a model of kinetics and other extensions, can be used to simulate complicated problems in self-assembly. This approach allows for a computationally tractable molecular dynamics-like simulation of coat protein interactions while retaining many relevant features of capsid self-assembly. Three simple simulation experiments are presented to illustrate the use of this model. These show the dependence of growth and malformation rates on the energetics of binding interactions, the tolerance of errors in binding positions, and the concentration of subunits in the examples. These experiments demonstrate a tradeoff within the model between growth rate and fidelity of assembly for the three parameters. A detailed discussion of the computational model is also provided.

L3 ANSWER 14 OF 35 MEDLINE on STN

1999011463. PubMed ID: 9792844. Electrostatic interactions drive scaffolding/coat protein binding and procapsid maturation in bacteriophage P22. Parker M H; **Prevelige P E Jr**. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama, 35294, USA.) Virology, (1998 Oct 25) 250 (2) 337-49. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The first step in assembly of the bacteriophage P22 is the formation of a T=7 icosahedral "procapsid," the major components of which are the coat protein and an inner core composed of the scaffolding protein. Although not present in the mature virion, the scaffolding protein is required for procapsid assembly. Eleven amino-acid residues at the extreme carboxyl terminus of the scaffolding protein are required for binding to the coat protein, and upon deletion of these residues, approximately 20 additional residues become disordered. Sequence analysis and NMR data suggest that the 30 residues at the carboxyl terminus form a helix-loop-helix motif which is stabilized by interhelical hydrophobic interactions. This "coat protein recognition domain" presents an unusually high number of positively charged residues on one face, suggesting that electrostatic interactions between this domain and the coat protein may contribute to recognition and binding. We report here that high ionic strength (1 M NaCl) completely inhibited procapsid assembly in vitro. When scaffolding protein was added to empty procapsid "shells" of coat protein, 1 M NaCl partially inhibited the binding of scaffolding protein to the shells. This suggests that the positively charged coat protein recognition domain at the carboxyl terminus of the scaffolding protein binds to a negatively charged region on the coat protein. During DNA packaging, the scaffolding protein exits the procapsid; scaffolding protein exit is followed by the expansion of the procapsid into a mature capsid. Procapsid shells can be induced to undergo a similar expansion reaction in vitro by heating (45-70 degreesC); this process was also inhibited by 1 M NaCl. These results are consistent with a model in which negatively charged scaffold protein-binding domains in the coat proteins move apart during procapsid expansion; this relief of electrostatic repulsion could provide a driving force for expansion and subsequent maturation. High-salt concentrations would screen this repulsion, while packaging of DNA (a polyanion) in vivo may increase the instability of the procapsid enough to trigger its expansion.

L3 ANSWER 15 OF 35 MEDLINE on STN

1998374275. PubMed ID: 9707570. Mechanism of capsid maturation in a double-stranded DNA virus. Tuma R; **Prevelige P E Jr**; Thomas G J Jr. (Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri, Kansas City, MO 64110-2499, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1998 Aug 18) 95 (17) 9885-90. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Folding mechanisms of proteins incorporated within supramolecular assemblies, including viruses, are little understood and may differ fundamentally from folding mechanisms of small globular proteins. We describe a novel Raman dynamic probe of hydrogen-isotope exchange to investigate directly these protein folding/assembly pathways. The method is applied to subunit folding in assembly intermediates of the double-stranded DNA bacteriophage P22. The icosahedral procapsid-to-capsid maturation (shell expansion) of P22 is shown to be accompanied by a large increase in exchange protection of peptide beta-strands. The molecular mechanism of shell expansion involves unfolding of metastable tertiary structure to form more stable quaternary contacts and is governed by a surprisingly high activation energy. The results demonstrate that coat subunit folding and capsid expansion are strongly coupled processes. Subunit structure in the procapsid represents a late intermediate along the folding/assembly pathway to the mature capsid. Coupling of folding and assembly is proposed as a general pathway for the construction of supramolecular complexes.

L3 ANSWER 16 OF 35 MEDLINE on STN

1998346995. PubMed ID: 9680477. A helical coat protein recognition domain of the bacteriophage P22 scaffolding protein. Tuma R; Parker M H; Weigele P; Sampson L; Sun Y; Krishna N R; Casjens S; Thomas G J Jr; **Prevelige P E Jr**. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA.) Journal of molecular biology, (1998 Aug 7) 281 (1) 81-94. Journal code: 2985088R. ISSN: 0022-2836. Pub. country:

ENGLAND: United Kingdom. Language: English.

AB The scaffolding protein of bacteriophage P22 directs the assembly of an icosahedral procapsid, a metastable shell that is the precursor for DNA packaging. The full-length protein has been shown previously to exist in a monomer-dimer-tetramer equilibrium of elongated and predominantly alpha-helical molecules. Two deletion-mutant fragments of the scaffolding protein, comprising amino acid residues 141 to 303 and 141 to 292, respectively, have been constructed, overexpressed in Escherichia coli, and purified. Removal of residues 1 to 140 yields a protein that is assembly-active both in vitro and in vivo, while the removal of the C-terminal 11 residues (293 to 303) leads to complete loss of scaffolding activity. Sedimentation analysis reveals that both scaffolding fragments exist in a monomer-dimer equilibrium governed by apparent dissociation constants Kd(141-303)=640 microM and Kd(141-292)=880 microM. Tetramer formation is not observed for either fragment; thus, the tetramerization domain of the scaffolding subunit resides in the N-terminal portion of the polypeptide chain. Examination of both fragments by circular dichroism, Raman and NMR spectroscopies indicates a highly alpha-helical fold in each case. Nonetheless, pronounced differences are observed between spectral signatures of the two fragments. Notably, Raman spectra of fragments 141-292 and 141-303 indicate that elimination of residues 293 to 303 results in unfolding of an alpha-helical coat protein "recognition" domain encompassing about 20 to 30 residues. The thermostability of fragment 141-303, monitored over a wide concentration range by circular dichroism and Raman spectroscopy, indicates a broad denaturation transition for the monomeric (low concentration) form, while more cooperative unfolding is observed for the dimeric (high concentration) form. A lesser increase in cooperativity upon dimerization is obtained for fragment 141-292. Additionally, the C-terminal recognition domain constitutes the most stable and cooperative unit in the 141-303 fragment. Measurement of hydrogen-isotope exchange kinetics in scaffolding fragments by time-resolved Raman spectroscopy shows that the C terminus is the only protected segment of the polypeptide chain. On the basis of the measured hydrodynamic and spectroscopic properties, a domain structure is proposed for the scaffolding subunit. The roles of these domains in P22 procapsid assembly are discussed.

L3 ANSWER 17 OF 35 MEDLINE on STN

1998346994. PubMed ID: 9680476. Functional domains of bacteriophage P22 scaffolding protein. Parker M H; Casjens S; **Prevelige P E Jr.** (Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA.) Journal of molecular biology, (1998 Aug 7) 281 (1) 69-79. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Assembly of the bacteriophage P22 requires a 303 amino acid residue scaffolding protein. Two scaffolding protein deletion mutants, consisting of residues 141 to 303 and 141 to 292, have been described. We report here that the 141-303 fragment, but not the 141-292 fragment, promoted procapsid assembly in vitro, bound to preformed shells of coat protein, and bound to a coat protein affinity column. These findings suggest that the carboxyl-terminal half of the scaffolding protein is sufficient for promoting assembly, and that the 11 amino acid residues at the extreme carboxyl terminus are required for binding to the coat protein. Analysis of the products of in vitro assembly reactions suggests that the maximum amount of scaffolding protein that can pack into a procapsid is dictated by the internal volume of the procapsid rather than by a finite number of binding sites. However, when the amount of scaffolding protein was reduced to limiting values, both the wild-type protein and the 141-303 fragment assembled procapsids with the same number, rather than the same mass, of scaffolding protein molecules. When the 141-292 fragment was added to a mixture of coat and scaffolding proteins, the initial phase of procapsid assembly was inhibited, but the final yield and composition of the procapsids were not affected. Assembly by a covalent dimeric mutant scaffolding protein (R74C/L177I) was not inhibited by the 141-292 fragment, which suggests that the inhibition is due to the formation of

[illegible] scaffolding protein. The 141-303 fragment, which has less tendency to self-associate than the wild-type protein, formed aberrant species as well as normal procapsid-like particles when the rate of assembly was high, suggesting that scaffolding protein dimerization may play a role in ensuring fidelity of assembly. Alternatively, residues 1 to 140 may play a direct structural role in preventing inappropriate scaffolding/coat protein interactions.

L3 ANSWER 18 OF 35 MEDLINE on STN
1998148712. PubMed ID: 9487732. Inhibiting virus-capsid assembly by altering the polymerisation pathway. **Prevelige P E Jr.** (Department of Microbiology, University of Alabama at Birmingham 35294, USA.) Trends in biotechnology, (1998 Feb) 16 (2) 61-5. Ref: 26. Journal code: 8310903. ISSN: 0167-7799. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Virus capsids assemble through the repeated interaction of well-defined protein subunits in a highly specific process. Basic research into the mechanism of protein polymerisation and virus assembly suggest that inhibition of the protein-protein interactions necessary for assembly is a valid therapeutic strategy. Computer models of virus-capsid assembly have located vulnerable stages in assembly, and small-molecule inhibitors of virus assembly have been identified. The challenge will be identifying agents that block assembly with the required specificity.

L3 ANSWER 19 OF 35 MEDLINE on STN
97376631. PubMed ID: 9232659. Cloning, purification, and preliminary characterization by circular dichroism and NMR of a carboxyl-terminal domain of the bacteriophage P22 scaffolding protein. Parker M H; Jablonsky M; Casjens S; Sampson L; Krishna N R; **Prevelige P E Jr.** (Department of Microbiology, University of Alabama at Birmingham 35294, USA.) Protein science : a publication of the Protein Society, (1997 Jul) 6 (7) 1583-6. Journal code: 9211750. ISSN: 0961-8368. Pub. country: United States. Language: English.
AB Assembly of double-stranded DNA viruses and bacteriophages involves the polymerization of several hundred molecules of coat protein, directed by an internal scaffolding protein. A 163-amino acid carboxyl-terminal fragment of the 303-amino acid bacteriophage P22 scaffolding protein was cloned, overexpressed, and purified. This fragment is active in procapsid assembly reactions in vitro. The circular dichroism spectrum of the fragment, as well as the 1D-NMR and 15N-1H HSQC spectra of the uniformly-labeled protein, indicate that stable secondary structure elements are present. Determination of the three dimensional packing of these elements into the folded scaffolding protein fragment is underway. Structure-based drug design targeted at structural proteins required for viral assembly may have potential as a therapeutic strategy.

L3 ANSWER 20 OF 35 MEDLINE on STN
97315115. PubMed ID: 9171289. Bacteriophage P22 scaffolding protein forms oligomers in solution. Parker M H; Stafford W F 3rd; **Prevelige P E Jr.** (Dept. of Microbiology, Univ. of Alabama at Birmingham, 35294, USA.) Journal of molecular biology, (1997 May 9) 268 (3) 655-65. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The scaffolding protein of Salmonella typhimurium bacteriophage P22 is a 33.6 kDa protein required both in vivo and in vitro for the polymerization of the viral coat protein into closed T = 7 icosahedral procapsids. In vitro assembly reaction kinetics have previously been found to vary between second and third order with respect to scaffolding protein concentration, suggesting that dimers and/or higher-order oligomers may be the active species in assembly. Analytical ultracentrifugation experiments suggest that scaffolding protein undergoes a rapidly-reversible monomer/dimer/tetramer equilibrium, with higher association constants at 4 degrees C than at 20 degrees C. Under conditions in which in vitro assembly reactions are carried out (30 to 1000 microg/ml scaffolding protein, 20 degrees C), monomers are the

predominant species, but the concentration of dimers is significant. A mutant scaffolding protein, R74C/L177I, which forms disulfide-linked dimers, catalyzed procapsid assembly at a higher rate than did the wild-type scaffolding protein; preincubation in dithiothreitol had little effect on the wild-type protein, but greatly reduced the activity of the mutant. These findings suggest that dimers and/or higher-order oligomers of scaffolding protein are active species in the assembly of P22.

L3 ANSWER 21 OF 35 MEDLINE on STN
96291577. PubMed ID: 8728649. The use of hydrostatic pressure as a tool to study viruses and other macromolecular assemblages. Silva J L; Foguel D; Da Poian A T; **Prevelige P E**. (Departamento de Bioquimica Medica, Universidade Federal do Rio de Janeiro, Brazil.. jerson@server.bioqmed.ufrj.br) . Current opinion in structural biology, (1996 Apr) 6 (2) 166-75. Ref: 79. Journal code: 9107784. ISSN: 0959-440X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Recent studies on the effect of pressure on macromolecular assemblages have provided new information on protein-protein and protein-nucleic acid interactions. New findings have recently emerged on the use of hydrostatic pressure to assess intermediate states in the assembly pathways of viruses, multimeric proteins and protein-nucleic acid complexes, addressing many questions of macromolecular recognition.

L3 ANSWER 22 OF 35 MEDLINE on STN
96283404. PubMed ID: 8676394. Three-dimensional structure of scaffolding-containing phage p22 procapsids by electron cryo-microscopy. Thuman-Commike P A; Greene B; Jakana J; Prasad B V; King J; **Prevelige P E Jr**; Chiu W. (Program in Structural & Computational Biology & Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030, USA.) Journal of molecular biology, (1996 Jul 5) 260 (1) 85-98. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The procapsids of bacterial viruses are the products of the polymerization of coat and scaffolding subunits, as well as the precursors in DNA packaging. Electron cryo-microscopy has been used to study the three-dimensional structures of bacteriophage P22 procapsids containing wild-type and mutant scaffolding proteins. The scaffolding mutant structure has been resolved to 19 A resolution and agrees with the 22 A resolution wild-type procapsid reconstruction. Both procapsid reconstructions contain an outer icosahedral coat protein shell and an inner scaffolding protein core. The outer core protein forms a T = 7 icosahedral lattice with distinctive channels present at the centers of the pentons and hexons. In addition, the hexons display a prominent skew. Computational isolation of the skewed hexon shows the presence of a local 2-fold axis that reduces the number of unique conformations in the asymmetric unit to four at this resolution. We have classified the four unique subunits into three distinct classes, based upon the shape of the upper domain and the presence of a channel leading to the inner coat protein surface. In addition, at the inner surface of the coat protein, finger-like regions that extend towards the scaffolding protein core are present in two of the subunits. The finger-like regions suggest the presence of an ordered interaction between the inner coat protein and the scaffolding protein. However, an icosahedral scaffolding protein shell is not formed, and the innermost scaffolding protein core does not pack with icosahedral symmetry.

L3 ANSWER 23 OF 35 MEDLINE on STN
96183325. PubMed ID: 8605213. Structural transitions in the scaffolding and coat proteins of P22 virus during assembly and disassembly. Tuma R; **Prevelige P E Jr**; Thomas G J Jr. (Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri- Kansas City 64110-2499, USA.) Biochemistry, (1996 Apr 9) 35 (14) 4619-27. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB An in vitro system for investigating the assembly of the Salmonella phage P22 has been exploited to elucidate the structural basis of recognition between scaffolding protein (gp8) and coat protein (gp5) subunits of the

viral procapsid. Raman spectroscopy and circular dichroism have been employed to examine structural thermostabilities of both gp8 and gp5 in native procapsids, and to characterize structural changes accompanying scaffolding exit, procapsid expansion, and shell disassembly. It is found that the secondary structure of the isolated gp8 subunit is rich in alpha-helix (approximately 40%), is highly thermolabile, and is characterized by noncooperative unfolding (Tm approximately 49 degrees C). Conversely, the procapsid-bound gp8 subunit exhibits stabilization of its alpha-helical secondary structure, characterized by cooperative unfolding. Because cooperative unfolding of gp8 coincides with exit from the procapsid, the present results suggest that unfolding and release are coupled processes. Structural differences between procapsid-free and procapsid-bound gp8 subunits are also apparent in Raman markers which monitor environments of tyrosine and tryptophan side chains. Temperature-resolved Raman spectroscopy of the empty procapsid shell reveals three distinct structural transitions for the gp5 subunits. The first, which occurs between 50 and 65 degrees C, is attributed to shell expansion and results in an increase in beta-strand secondary structure. The two higher temperature transitions, occurring within intervals of 70-80 and 80-95 degrees C, respectively, are attributed to partial unfolding of the shell subunit and subsequent shell disassembly. The same gp5 structure transitions are detected for procapsids which contain scaffolding protein. On the basis of the observed thermodynamic coupling between gp8 unfolding and its release from the procapsid, we propose a model for P22 procapsid assembly. Implications of the model for in vivo assembly of dsDNA viruses are discussed.

L3 ANSWER 24 OF 35 MEDLINE on STN

95127681. PubMed ID: 7827060. Role of entropic interactions in viral capsids: single amino acid substitutions in P22 bacteriophage coat protein resulting in loss of capsid stability. Foguel D; Teschke C M; **Prevelige P E Jr**; Silva J L. (Departamento de Bioquimica, Universidade Federal do Rio de Janeiro, Brazil.) Biochemistry, (1995 Jan 31) 34 (4) 1120-6. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Bacteriophage P22 is a double-stranded DNA containing phage. Its morphogenetic pathway requires the formation of a precursor procapsid that subsequently matures to the capsid. The stability of bacteriophage P22 coat protein in both monomeric and polymeric forms under hydrostatic pressure has been examined previously [Prevelige, P. E., King, J., & Silva, J. L. (1994) Biophys. J. 66, 1631-1641]. The monomeric protein is very unstable to pressure and undergoes denaturation at pressures below 1.5 kbar, whereas the procapsid shell is very stable to applied pressure and does not dissociate with pressure to 2.5 kbar. However, under applied pressure the procapsid shells are cold labile, suggesting they are entropically stabilized. We have analyzed the pressure stability of mutant procapsid shells having either of two single amino acid substitutions in the coat protein (G232D and W48Q) using light-scattering and fluorescence emission methods. While the wild-type shells were stable under 2.2 kbar of pressure at room temperature (22 degrees C), the G232D mutant shells showed time-dependent dissociation under these conditions. Decreasing the temperature to 1 degree C dramatically accelerated the dissociation of G232D mutant under applied pressure. On the other hand, the W48Q mutant shells could be dissociated easily by pressure at room temperature and displayed little dependence on temperature, suggesting a smaller entropic contribution to the stability of this mutant. The unpolymerized mutant subunits displayed a pressure stability similar to that of the wild type.(ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 25 OF 35 MEDLINE on STN

94339362. PubMed ID: 8061212. Pressure denaturation of the bacteriophage P22 coat protein and its entropic stabilization in icosahedral shells. **Prevelige P E Jr**; King J; Silva J L. (Boston Biomedical Research Institute, Massachusetts 02114.) Biophysical journal, (1994 May) 66 (5) 1631-41. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB The pressure stability of bacteriophage P22 coat protein in both monomeric and polymeric forms under hydrostatic pressure was examined using light scattering, fluorescence emission, polarization, and lifetime methodology. The monomeric protein is very unstable toward pressure and undergoes significant structural changes at pressures as low as 0.5 kbar. These structural changes ultimately lead to denaturation of the subunit. Comparison of the protein denatured by pressure to that in guanidine hydrochloride suggests that pressure results in partial unfolding, perhaps by a domain mechanism. Fluorescence lifetime measurements indicate that at atmospheric pressure the local environments of the tryptophans are remarkably similar, suggesting they may be clustered. In contrast to the monomeric protein subunit, the protein when polymerized into procapsid shells is very stable to applied pressure and does not dissociate with pressure up to 2.5 kbar. However, under applied pressure the procapsid shells are cold-labile, suggesting they are entropically stabilized. The significance of these results in terms of virus assembly are discussed.

L3 ANSWER 26 OF 35 MEDLINE on STN

94002057. PubMed ID: 8399211. Inhibition of viral capsid assembly by 1,1'-bi(4-anilinonaphthalene-5-sulfonic acid). Teschke C M; King J; **Prevelige P E Jr**. (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.) Biochemistry, (1993 Oct 12) 32 (40) 10658-65. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The precursor shells of dsDNA bacteriophages are assembled by the polymerization of competent states of coat and scaffolding subunits. The fluorescent dye 1,1'-bi(4-anilinonaphthalene-5-sulfonic acid) (bisANS) binds to both the coat and scaffolding proteins from the Salmonella typhimurium bacteriophage P22. It displays little affinity for the polymerized forms of the proteins. The subunits with bound bisANS are incapable of assembling into procapsids. The binding constants of bisANS for both coat and scaffolding protein monomers have been measured and are 7 and 6 microM, respectively. Binding of bisANS to coat protein has little effect on the conformation as determined by circular dichroism and susceptibility to proteolysis. Binding of bisANS to scaffolding protein induces a change in the secondary structure consistent with a loss of alpha-helix, and an altered susceptibility to proteolysis. We suggest that the bisANS is probably binding at sites responsible for intersubunit interactions and thereby inhibiting capsid assembly.

L3 ANSWER 27 OF 35 MEDLINE on STN

93267654. PubMed ID: 8496966. Three-dimensional transformation of capsids associated with genome packaging in a bacterial virus. Prasad B V; **Prevelige P E**; Marietta E; Chen R O; Thomas D; King J; Chiu W. (Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030.) Journal of molecular biology, (1993 May 5) 231 (1) 65-74. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Three-dimensional structures of the empty procapsid and the mature capsid of the Salmonella bacteriophage P22 have been determined to a resolution of 28 A using electron cryomicroscopy and computer image processing. The coat subunits in both the structures are arranged as pentamers and hexamers on a T = 7 icosahedral lattice. The two structures display significant differences in shape, size and intersubunit interactions. The empty procapsid is spherical in contrast to the distinctly larger and polyhedral mature capsid. The empty procapsid structure exhibits holes at all the quasi sixfold positions that are absent in the mature capsid. These holes may be the exit ports for scaffolding subunits. Detailed comparisons of the two structures indicate that extensive structural changes take place during maturation in all seven quasi-equivalent subunits. These changes cause flattening of the icosahedral facets, capsid expansion and closing of the holes. This process results in a stable and impenetrable capsid that protects the bacterial genome.

L3 ANSWER 28 OF 35 MEDLINE on STN

93229690. PubMed ID: 8471727. Nucleation and growth phases in the

polymerization of coat and scaffolding subunits into icosahedral procapsid shells. **Prevelige P E Jr;** Thomas D; King J. (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.) Biophysical journal, (1993 Mar) 64 (3) 824-35. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB The polymerization of protein subunits into precursor shells empty of DNA is a critical process in the assembly of double-stranded DNA viruses. For the well-characterized icosahedral procapsid of phage P22, coat and scaffolding protein subunits do not assemble separately but, upon mixing, copolymerize into double-shelled procapsids in vitro. The polymerization reaction displays the characteristics of a nucleation limited reaction: a paucity of intermediate assembly states, a critical concentration, and kinetics displaying a lag phase. Partially formed shell intermediates were directly visualized during the growth phase by electron microscopy of the reaction mixture. The morphology of these intermediates suggests that assembly is a highly directed process. The initial rate of this reaction depends on the fifth power of the coat subunit concentration and the second or third power of the scaffolding concentration, suggesting that pentamer of coat protein and dimers or trimers of scaffolding protein, respectively, participate in the rate-limiting step.

L3 ANSWER 29 OF 35 MEDLINE on STN

93174008. PubMed ID: 8438077. Assembly of bacteriophage P22: a model for ds-DNA virus assembly. **Prevelige P E Jr;** King J. (Department of Biology, Massachusetts Institute of Technology, Cambridge.) Progress in medical virology. Fortschritte der medizinischen Virusforschung. Progres en virologie medicale, (1993) 40 206-21. Journal code: 0376451. ISSN: 0079-645X. Pub. country: Switzerland. Language: English.

L3 ANSWER 30 OF 35 MEDLINE on STN

93136147. PubMed ID: 8422364. Subunit conformational changes accompanying bacteriophage P22 capsid maturation. **Prevelige P E Jr;** Thomas D; Aubrey K L; Towse S A; Thomas G J Jr. (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.) Biochemistry, (1993 Jan 19) 32 (2) 537-43. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB In double-stranded DNA bacteriophages, packaging of dsDNA requires the transformation of a precursor procapsid into a mature viral capsid. Lattice expansion and release of scaffolding subunits accompanying DNA packaging. Three-dimensional structures of procapsid and mature phage lattices demonstrate that the capsid transformation involves substantial changes in subunit environment. Since this transformation occurs without subunit dissociation, it represents a transition between at least two stable subunit conformations. Using Raman spectroscopy, we have identified changes in coat protein secondary structure and side-chain environments which accompany the capsid transformation. The subunits of procapsid shells contain only 2.0 +/- 0.4% more alpha-helix and less beta-sheet than those of mature capsids; however, numerous side chains are substantially altered by the transformation, including tyrosines, tryptophans, phenylalanines, and aliphatics, which are widely distributed through the subunit sequence. We propose, therefore, that procapsid expansion is accomplished through the relative motion of coat subunit domains with little change in secondary structure. Such hinge-bending conformational transitions may couple ATP-dependent dsDNA condensation with shell expansion.

L3 ANSWER 31 OF 35 MEDLINE on STN

91220717. PubMed ID: 2024494. A pilot protein participates in the initiation of P22 procapsid assembly. Thomas D; **Prevelige P Jr.** (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.) Virology, (1991 Jun) 182 (2) 673-81. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The gene 16 protein of the bacteriophage P22 is required as a pilot protein aiding the transfer of DNA from the phage into the Salmonella typhimurium host cell. During assembly 10-20 copies of the 63,000-Da gp 16 protein are incorporated into the procapsid shell prior to DNA

packaging. The protein has been purified from isolated procapsids and behaved as a monomer in solution. Upon incubation with purified coat and scaffolding subunits in vitro, it assembled into procapsids with the correct stoichiometry. The addition of physiological quantities of gp 16 resulted in an increased rate of procapsid assembly. Sedimentation of mixtures of coat and gp 16 protein subunits revealed association/dissociation behavior. It is likely that the added gp 16 is acting to stabilize a transient oligomeric coat protein species that functions as the in vitro initiation complex for procapsid assembly.

L3 ANSWER 32 OF 35 MEDLINE on STN

90352317. PubMed ID: 2386790. Conformational states of the bacteriophage P22 capsid subunit in relation to self-assembly. **Prevelige P E Jr;** Thomas D; King J; Towse S A; Thomas G J Jr. (Division of Cell Biology and Biophysics, School of Basic Life Sciences, University of Missouri-Kansas City 64110.) Biochemistry, (1990 Jun 12) 29 (23) 5626-33. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The formation of closed icosahedral capsids from a single species of coat protein subunit requires that the subunits assume different conformations at different lattice positions. In the double-stranded DNA bacteriophage P22, formation of correctly dimensioned capsids is mediated by interaction between coat protein subunits and scaffolding protein. Raman spectroscopy has been employed to compare the conformations of coat protein subunits which have been polymerized to form capsids in the presence and absence of the of scaffolding protein display a Raman spectrum characterized by a broad amide I band centered at 1665 cm^{-1} with a discernible shoulder near 1653 cm^{-1}, and a broad amide III profile centered at 1238 cm^{-1} but asymmetrically skewed to higher frequency. These spectral features indicate that the protein conformation in procapsid shells is rich in beta-sheet secondary structure but contains also a significant distribution of alpha-helix. When biologically active, purified subunits assemble in the absence of scaffolding protein, they form polydisperse multimers lacking the proper dimensions of procapsid closed shells. We designate these multimers as "associated subunits" (AS). The Raman spectrum of associated subunits indicates a narrower distribution of secondary structure. The associated subunits are characterized by a sharper and more intense Raman amide I band at 1666 cm^{-1}, with no prominent amide I shoulder of lower frequency. An analogous narrowing of the Raman amide III profile is also observed for AS particles, with an accompanying shift of the amide III band center to 1235 cm^{-1}.(ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 33 OF 35 MEDLINE on STN

89011999. PubMed ID: 3262767. Scaffolding protein regulates the polymerization of P22 coat subunits into icosahedral shells in vitro. **Prevelige P E Jr;** Thomas D; King J. (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.) Journal of molecular biology, (1988 Aug 20) 202 (4) 743-57. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Coat and scaffolding subunits derived from P22 procapsids have been purified in forms that co-assemble rapidly and efficiently into icosahedral shells in vitro under native conditions. The half-time for this reaction is approximately five minutes at 21 degrees C. The in vitro reaction exhibits the regulated features observed in vivo. Neither coat nor scaffolding subunits alone self-assemble into large structures. Upon mixing the subunits together they polymerize into procapsid-like shells with the in vivo coat and scaffolding protein composition. The subunits in the purified coat protein preparations are monomeric. The scaffolding subunits appear to be monomeric or dimeric. These results confirm that P22 procapsid formation does not proceed through the assembly of a core of scaffolding, which then organizes the coat, but requires copolymerization of coat and scaffolding. To explore the mechanisms of the control of polymerization, shell assembly was examined as a function of the input ratio of scaffolding to coat subunits. The results indicated that scaffolding protein was required for both initiation of shell assembly and continued polymerization. Though procapsids produced in vivo contain

about 300 molecules of scaffolding, shells with fewer subunits could be assembled down to a lower limit of about 140 scaffolding subunits per shell. The overall results of these experiments indicate that coat and scaffolding subunits must interact in both the initiation and the growth phases of shell assembly. However, it remains unclear whether during growth the coat and scaffolding subunits form a mixed oligomer prior to adding to the shell or whether this occurs at the growing edge.

L3 ANSWER 34 OF 35 MEDLINE on STN

87271588. PubMed ID: 3607000. Structural studies of acetylated and control inner core histones. **Prevelige P E Jr**; Fasman G D. Biochemistry, (1987 May 19) 26 (10) 2944-55. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The role of acetylation on the conformation and association state of the inner core histone octamer isolated from HeLa cells was examined. Preparation of suitable quantities of pure acetylated and control inner core histones from HeLa cells required the development of a new preparative procedure. The results from size-exclusion high-performance liquid chromatography and sedimentation equilibrium studies indicated that acetylated inner core histones associate to species larger than the octamer and form a more stable complex. Circular dichroism studies demonstrated that the amount of alpha-helix increases with increasing association of the histones. Furthermore, acetylation results in an increase in the amount of alpha-helix, perhaps coupled through its effect on the association state. At high protein concentration and elevated temperature, the acetylated sample displays a greater increase in beta-sheet content, relative to the control sample. This increase in beta-sheet content may be induced during the association of the acetylated sample to species larger than the octamer. There is a marked effect on the conformation of both acetylated and control inner core histones as a function of protein concentration, ionic strength, and temperature. The difference in conformational flexibility and association state of the acetylated vs. the control inner histone core may play a significant role in the control of transcription in the nucleus.

L3 ANSWER 35 OF 35 MEDLINE on STN

83153699. PubMed ID: 6830804. Studies on synthetic chromatins containing poly(dA-dT) X poly(dA-dT) and poly(dG-dC) X poly(dG-dC). **Prevelige P E Jr**; Fasman G D. Biochimica et biophysica acta, (1983 Jan 20) 739 (1) 85-96. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Core histones (H2A,H2B,H3,H4)2, were reconstituted with the synthetic polynucleotides poly(dA-dT) X poly(dA-dT) and poly(dG-dC) X poly(dG-dC) to yield synthetic chromatins containing 200 basepairs per octamer. These synthetic chromatins displayed a 36% decrease in the circular dichroism (CD) peak ellipticity from the value of the polynucleotide free in solution; the poly(dA-dT) X poly(dA-dT)/chromatin showed an increase in the complexity of the thermal denaturation profile compared to that of the polynucleotide. Both the temperature of maximum dh/dT for each transition (Tm) and the relative amount of poly(dA-dT) X poly(dA-dT) in the synthetic chromatin melting in each of the four thermal transitions is a function of the ionic strength over the 0-5 mM sodium phosphate range (0.25 mM EDTA, pH 7.0); a shift of material toward higher melting transitions was observed with increasing ionic strength. The CD peak ellipticity value for both synthetic chromatins was ionic strength-independent over the 0-5 mM sodium phosphate range. These results are in contrast to those observed with H1/H5 stripped chicken erythrocyte chromatin (Fulmer, A. and Fasman, G.D. (1979) Biopolymers 18, 2875-2891), where an ionic strength dependence was found. Differences in the CD spectra between poly(dA-dT) X poly(dA-dT)/chromatin, poly(dG-dC) X poly(dG-dC)/chromatin and H1/H5 stripped chicken erythrocyte chromatin suggest subtle differences in assembly. Finally, the temperature dependence of the CD spectra of poly(dA-dT) X poly(dA-dT)-containing synthetic chromatin, which is similar to that for the polynucleotide, suggests the core histone bound polynucleotide has a large degree of conformational flexibility allowing it to undergo the premelt transition.

```
=> d his

     (FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

     FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
               E PREVELIGE P/AU

     FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1             1 S US5716613/PN
L2             1 S US5789245/PN

     FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
               E PREVELIGE P E/AU
L3            35 S E3 OR E4 OR E5

=>
=> e klishko v y/au
E1             1     KLISHKO O K/AU
E2             1     KLISHKO V/AU
E3             2 --> KLISHKO V Y/AU
E4             1     KLISHKO VYU/AU
E5            43     KLISHOV A A/AU
E6             3     KLISHOVA Z N/AU
E7             3     KLISIARIS VASILIOS K/AU
E8             3     KLISIC JELENA/AU
E9             3     KLISIC L/AU
E10           30     KLISIC P/AU
E11            3     KLISIC P J/AU
E12           14     KLISIECKI A/AU

=> s e2-e4
               1 "KLISHKO V"/AU
               2 "KLISHKO V Y"/AU
               1 "KLISHKO VYU"/AU
L4             4 ("KLISHKO V"/AU OR "KLISHKO V Y"/AU OR "KLISHKO VYU"/AU)

=> d l4,cbib,ab,1-4

L4   ANSWER 1 OF 4     MEDLINE on STN
1999102696.    PubMed ID: 9872746.   Assembly and analysis of conical models
     for the HIV-1 core. Ganser B K; Li S; Klishko V Y; Finch J T; Sundquist
     W I. (Department of Biochemistry, University of Utah, Salt Lake City, UT
     84132, USA. ) Science, (1999 Jan 1) 283 (5398) 80-3.  Journal code:
     0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.
AB   The genome of the human immunodeficiency virus (HIV) is packaged within an
     unusual conical core particle located at the center of the infectious
     virion.  The core is composed of a complex of the NC (nucleocapsid)
     protein and genomic RNA, surrounded by a shell of the CA (capsid) protein.
     A method was developed for assembling cones in vitro using pure
     recombinant HIV-1 CA-NC fusion proteins and RNA templates.  These
     synthetic cores are capped at both ends and appear similar in size and
     morphology to authentic viral cores.  It is proposed that both viral and
     synthetic cores are organized on conical hexagonal lattices, which by
     Euler's theorem requires quantization of their cone angles.  Electron
     microscopic analyses revealed that the cone angles of synthetic cores were
     indeed quantized into the five allowed angles.  The viral core and most
     synthetic cones exhibited cone angles of approximately 19 degrees (the
     narrowest of the allowed angles).  These observations suggest that the
     core of HIV is organized on the principles of a fullerene cone, in analogy
     to structures recently observed for elemental carbon.

L4   ANSWER 2 OF 4     MEDLINE on STN
1998169372.    PubMed ID: 9501077.   Proteolytic refolding of the HIV-1 capsid
     protein amino-terminus facilitates viral core assembly. von Schwedler U K;
```

[redacted] I. (Department of Biochemistry, University of Utah, Salt Lake City, UT 84132, USA.) EMBO journal, (1998 Mar 16) 17 (6) 1555-68. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB After budding, the human immunodeficiency virus (HIV) must 'mature' into an infectious viral particle. Viral maturation requires proteolytic processing of the Gag polyprotein at the matrix-capsid junction, which liberates the capsid (CA) domain to condense from the spherical protein coat of the immature virus into the conical core of the mature virus. We propose that upon proteolysis, the amino-terminal end of the capsid refolds into a beta-hairpin/helix structure that is stabilized by formation of a salt bridge between the processed amino-terminus (Pro1) and a highly conserved aspartate residue (Asp51). The refolded amino-terminus then creates a new CA-CA interface that is essential for assembling the condensed conical core. Consistent with this model, we found that recombinant capsid proteins with as few as four matrix residues fused to their amino-termini formed spheres in vitro, but that removing these residues refolded the capsid amino-terminus and redirected protein assembly from spheres to cylinders. Moreover, point mutations throughout the putative CA-CA interface blocked capsid assembly in vitro, core assembly in vivo and viral infectivity. Disruption of the conserved amino-terminal capsid salt bridge also abolished the infectivity of Moloney murine leukemia viral particles, suggesting that lenti- and oncoviruses mature via analogous pathways.

L4 ANSWER 3 OF 4 MEDLINE on STN

90148505. PubMed ID: 2619935. Variance of writhe for wormlike DNA rings with excluded volume. Klenin K V; Vologodskii A V; Anshelevich V V; **Klishko VYu**; Dykhne A M; Frank-Kamenetskii M D. (Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow.) Journal of biomolecular structure & dynamics, (1989 Feb) 6 (4) 707-14. Journal code: 8404176. ISSN: 0739-1102. Pub. country: United States. Language: English.

AB We have calculated the variance of the equilibrium distribution of a circular wormlike polymer chain over the writhing number, less than (Wr)2 greater than, with allowance for the excluded volume effects. Within this model the less than (Wr)2 greater than value is a function of the number of Kuhn statistical segments, n, and the chain diameter, d measured in Kuhn statistical lengths, b. Simulated DNA chains varied from 200 to 10,000 base pairs and the d value varied from 0.02 to 0.2. Theory predicts a considerable ionic strength dependence of the DNA superhelix energy as a consequence of the change in the DNA diameter. A comparison with the available experimental data has yielded an estimate of the DNA torsional rigidity, the Kuhn statistical length, and the effective diameter of the double helix under conditions of the complete screening of the DNA electrostatic potential.

L4 ANSWER 4 OF 4 MEDLINE on STN

90121821. PubMed ID: 6400822. Kinetics of cruciform formation and stability of cruciform structure in superhelical DNA. Panyutin I; **Klishko V**; Lyamichev V. (Institute of Molecular Genetics Academy of Sciences of the U.S.S.R. Moscow.) Journal of biomolecular structure & dynamics, (1984 Jun) 1 (6) 1311-24. Journal code: 8404176. ISSN: 0739-1102. Pub. country: United States. Language: English.

AB This is a study of the kinetics of formation of a cruciform structure from the longest palindromic sequence in plasmid pAO3 DNA. DNA was prepared so as to be free of cruciforms even in topoisomers whose negative superhelicity was great enough to induce cruciform formation. Samples of such DNA were incubated at various temperatures, the incubation time varying over a wide range. Then the state was frozen by chilling. Two-dimensional electrophoretic analysis made it possible to estimate the fraction of molecules that got the cruciform structure during incubation. Precautions were taken for electrophoresis conditions to rule out any spontaneous conformational changes within the palindromic region. The relaxation time at the midpoint of the transition ranged from 30 min at 30 C to 50 hrs at 20 C, both in 0.1SSC. An increase in the negative

supercritical density by 3.81 led to a 300 fold reduction of the relaxation time at 30 C but had little effect at 20 C. The probability of cruciform formation has been examined as a function of temperature. It has been shown that the cruciform state is no longer the predominant one at elevated temperatures: the cruciformation probability drops to an insignificant value for all of the topoisomers involved. Data have been obtained suggesting that the cruciform formation at the major palindromic site is not the only structural transition possible in pAO3 DNA.

```
=>
=> e gross i/au
E1            1      GROSS HILLERY/AU
E2            2      GROSS HORST/AU
E3           85 --> GROSS I/AU
E4            1      GROSS I F/AU
E5            1      GROSS I M/AU
E6            2      GROSS IAN/AU
E7            3      GROSS ISABELLE/AU
E8           27      GROSS ISSEROFF R/AU
E9            4      GROSS ISSEROFF RUTH/AU
E10           1      GROSS IU/AU
E11           2      GROSS IU R/AU
E12           2      GROSS IWONA/AU

=> e hohenberg h/au
E1            2      HOHENBERG GERDA/AU
E2            1      HOHENBERG GREGOR/AU
E3           36 --> HOHENBERG H/AU
E4            3      HOHENBERG HEINZ/AU
E5            1      HOHENBERG J K/AU
E6            1      HOHENBERG P C/AU
E7            2      HOHENBERGER B/AU
E8            6      HOHENBERGER E/AU
E9            1      HOHENBERGER I/AU
E10           1      HOHENBERGER J/AU
E11           2      HOHENBERGER K/AU
E12           4      HOHENBERGER M/AU

=> s e3 or e4
             36 "HOHENBERG H"/AU
              3 "HOHENBERG HEINZ"/AU
L5           39 "HOHENBERG H"/AU OR "HOHENBERG HEINZ"/AU

=> d l5,cbib,ab,1-39

L5   ANSWER 1 OF 39     MEDLINE on STN
2004154919.    PubMed ID: 15047813.   Spread of hepatitis B viruses in vitro
     requires extracellular progeny and may be codetermined by polarized
     egress. Funk A; Hohenberg H; Mhamdi M; Will H; Sirma H. (Department of
     General Virology, Heinrich-Pette-Institut fur experimentelle Virologie und
     Immunologie an der Universitat Hamburg, 20251 Hamburg, Germany. ) Journal
     of virology, (2004 Apr) 78 (8) 3977-83.  Journal code: 0113724. ISSN:
     0022-538X. Pub. country: United States. Language: English.
AB   Viruses can spread by different mechanisms: via intracellular particles
     through cell junctions to neighboring cells or via secreted virions to
     adjacent or remote cells.  The observation of clusters of
     hepadnavirus-infected cells both in vivo and in primary hepatocytes
     neither proves the first mechanism nor excludes the second.  In order to
     test which mechanism, if not both, is used by hepatitis B viruses in order
     to spread, we used primary duck hepatocytes and duck hepatitis B virus
     (DHBV) as an infection model.  If extracellular progeny virus alone
     determines spreading, neutralizing antisera or drugs blocking virus
     binding to hepatocytes should abolish secondary infection.  In order to
     test this, we used DHBV envelope-specific neutralizing antisera, as well
     as suramin, a known inhibitor of infection.  Both reagents strongly
```

reduced hepatocellular detachment of viral particles and almost completely abolished primary infection, whereas an ongoing intracellular infection was not affected as long as no progeny virus was released. In contrast, incubation of infected primary hepatocytes with these reagents during release of progeny virus completely prevented secondary infection. Moreover, the combination of electron and immunofluorescence microscopy analyses revealed the residence of viral particles in cytoplasmic vesicles preferentially located near the basolateral membrane of infected hepatocytes. Taken together, these data strongly suggest that hepatitis B viruses mainly spread by secreted, extracellular progeny and point to polarized egress of viral particles into intercellular compartments, which restricts their diffusion and favors transmission of virus to adjacent cells.

L5 ANSWER 2 OF 39 MEDLINE on STN

2003497544. PubMed ID: 14516364. Cryoimmobilization and three-dimensional visualization of C. elegans ultrastructure. Muller-Reichert T; **Hohenberg H**; O'Toole E T; McDonald K. (Max Planck Institute for Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, D-01307 Dresden, Germany.. mueller.reichart@mpi-cbg.de) . Journal of microscopy, (2003 Oct) 212 (Pt 1) 71-80. Journal code: 0204522. ISSN: 0022-2720. Pub. country: England: United Kingdom. Language: English.

AB Caenorhabditis elegans is one of the most important genetic systems used in current biological research. Increasingly, these genetics-based research projects are including ultrastructural analyses in their attempts to understand the molecular basis for cell function. Here, we present and review state-of-the-art methods for both ultrastructural analysis and immunogold localization in C. elegans. For the initial cryofixation, high-pressure freezing is the method of choice, and in this article we describe two different strategies to prepare these nematode worms for rapid freezing. The first method takes advantage of transparent, porous cellulose capillary tubes to contain the worms, and the second packs the worms in E. coli and/or yeast paste prior to freezing. The latter method facilitates embedding of C. elegans in a thin layer of resin so individual worms can be staged, selected and precisely orientated for serial sectioning followed by immunolabelling or electron tomography.

L5 ANSWER 3 OF 39 MEDLINE on STN

2003120797. PubMed ID: 12634782. Self-organization of dissolved organic matter to micelle-like microparticles in river water. Kerner Martin; **Hohenberg Heinz**; Ertl Siegmund; Reckermann Marcus; Spitzy Alejandro. (University of Hamburg, Institute for Hydrobiology and Fishery Science, D-22765 Hamburg, Zeiseweg 9, Germany.. kerner@uni-hamburg.de) . Nature, (2003 Mar 13) 422 (6928) 150-4. Journal code: 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

AB In aquatic systems, the concept of the 'microbial loop' is invoked to describe the conversion of dissolved organic matter to particulate organic matter by bacteria. This process mediates the transfer of energy and matter from dissolved organic matter to higher trophic levels, and therefore controls (together with primary production) the productivity of aquatic systems. Here we report experiments on laboratory incubations of sterile filtered river water in which we find that up to 25% of the dissolved organic carbon (DOC) aggregates abiotically to particles of diameter 0.4-0.8 micrometres, at rates similar to bacterial growth. Diffusion drives aggregation of low- to high-molecular-mass DOC and further to larger micelle-like microparticles. The chemical composition of these microparticles suggests their potential use as food by planktonic bacterivores. This pathway is apparent from differences in the stable carbon isotope compositions of picoplankton and the microparticles. A large fraction of dissolved organic matter might therefore be channelled through microparticles directly to higher trophic levels--bypassing the microbial loop--suggesting that current concepts of carbon conversion in aquatic systems require revision.

L5 ANSWER 4 OF 39 MEDLINE on STN

2003019234. PubMed ID: 12525630. New hepatitis B virus of cranes that has

Kalinina Tatyana; Schneider Carola; Cova Lucyna; Krone Oliver; Frolich Kai; Will Hans; Sirma Huseyin. (Heinrich Pette Institute of Experimental Virology and Immunology, Hamburg. Institute of Zoo and Wildlife Research, Berlin, Germany.) Journal of virology, (2003 Feb) 77 (3) 1964-76. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB All hepadnaviruses known so far have a very limited host range, restricted to their natural hosts and a few closely related species. This is thought to be due mainly to sequence divergence in the large envelope protein and species-specific differences in host components essential for virus propagation. Here we report an infection of cranes with a novel hepadnavirus, designated CHBV, that has an unexpectedly broad host range and is only distantly evolutionarily related to avihepadnaviruses of related hosts. Direct DNA sequencing of amplified CHBV DNA as well a sequencing of cloned viral genomes revealed that CHBV is most closely related to, although distinct from, Ross' goose hepatitis B virus (RGHBV) and slightly less closely related to duck hepatitis B virus (DHBV). Phylogenetically, cranes are very distant from geese and ducks and are most closely related to herons and storks. Naturally occurring hepadnaviruses in the last two species are highly divergent in sequence from RGHBV and DHBV and do not infect ducks or do so only marginally. In contrast, CHBV from crane sera and recombinant CHBV produced from LMH cells infected primary duck hepatocytes almost as efficiently as DHBV did. This is the first report of a rather broad host range of an avihepadnavirus. Our data imply either usage of similar or identical entry pathways and receptors by DHBV and CHBV, unusual host and virus adaptation mechanisms, or divergent evolution of the host genomes and cellular components required for virus propagation.

L5 ANSWER 5 OF 39 MEDLINE on STN

2002698795. PubMed ID: 12460721. Distribution of sunscreens on skin. Schulz J; **Hohenberg H**; Pflucker F; Gartner E; Will T; Pfeiffer S; Wepf R; Wendel V; Gers-Barlag H; Wittern K-P. (R&D Cosmed, Beiersdorf AG, Function 4243, Unnastrasse 48, D-20245, Hamburg, Germany.. jens.schulz@beiersdorf.com) . Advanced drug delivery reviews, (2002 Nov 1) 54 Suppl 1 S157-63. Ref: 6. Journal code: 8710523. ISSN: 0169-409X. Pub. country: Netherlands. Language: English.

AB The effectiveness of sunscreens was originally achieved by incorporation of soluble organic UV absorbers such as cinnamates and others into cosmetic formulations. Determinations of the sun protection factor (SPF) of emulsions containing different organic UV absorbers clearly indicate that the efficacy depends on the absorption characteristics of each single UV filter substance. Nowadays, micronised pigments such as titanium dioxide or zinc oxide have also been found to be protective against harmful UV rays. Our investigations using optical and electron microscopy proved that neither surface characteristics, particle size nor shape of the micronised pigments result in any dermal absorption of this substance. Micronised titanium dioxide is solely deposited on the outermost surface of the stratum corneum and cannot be detected in deeper stratum corneum layers, the human epidermis and dermis.

L5 ANSWER 6 OF 39 MEDLINE on STN

2002695940. PubMed ID: 12457285. Efficient gene transfer into the CNS by lentiviral vectors purified by anion exchange chromatography. Scherr M; Battmer K; Eder M; Schule S; **Hohenberg H**; Ganser A; Grez M; Blomer U. (Department of Hemato-Oncology, Medizinische Hochschule Hannover, D-30625 Hannover, Germany.) Gene therapy, (2002 Dec) 9 (24) 1708-14. Journal code: 9421525. ISSN: 0969-7128. Pub. country: England: United Kingdom. Language: English.

AB Lentiviral vectors have been shown to stably transduce dividing and non-dividing target cells in vitro and in vivo. However, in vivo gene transfer applications with viral vectors in the central nervous system require highly efficient vector preparations, because only very small volumes can be injected stereotactically without damage to the brain tissue. Since lentiviral vectors are generated in transient

efficiently concentrated before injection into the brain. We describe an alternative procedure to concentrate lentiviral preparations by binding viral particles to an anion exchange column. Viral particles are eluted with sodium chloride, desalted and further concentrated by ultrafiltration. These vector preparations allowed high levels of gene transfer into terminally differentiated neuronal and glial cells and long-term transgene expression without any signs of acute and long-term toxicity or inflammation. The purification of lentiviral vectors from large-scale preparations by anion exchange chromatography allowed us to concentrate the virus to small volumes and to use these preparations to genetically modified target cells in vivo without signs of acute inflammatory responses.

L5 ANSWER 7 OF 39 MEDLINE on STN

2002149700. PubMed ID: 11880649. Antivirally active MxA protein sequesters La Crosse virus nucleocapsid protein into perinuclear complexes. Kochs Georg; Janzen Christian; **Hohenberg Heinz**; Haller Otto. (Abteilung Virologie, Institut fur Medizinische Mikrobiologie und Hygiene, Universitat Freiburg, D-79008 Freiburg, Germany.. kochs@ukl.uni-freiburg.de) . Proceedings of the National Academy of Sciences of the United States of America, (2002 Mar 5) 99 (5) 3153-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Bunyaviruses replicate in the cytoplasm of infected cells. New viral particles are formed by budding of nucleocapsids into the Golgi apparatus. We have previously shown that the IFN-induced human MxA protein inhibits bunyavirus replication by an unknown mechanism. Here we demonstrate that MxA binds to the nucleocapsid protein of La Crosse virus (LACV) and colocalizes with the viral protein in cytoplasmic complexes. Electron microscopy revealed that these complexes accumulated in the perinuclear area and consisted of highly ordered fibrillary structures. A similar MxA-mediated redistribution of viral nucleocapsid proteins was detected with other bunyaviruses, such as Bunyamwera virus and Rift Valley fever virus. MxA(E645R), a carboxy-terminal mutant of MxA without antiviral activity against LACV, did not lead to complex formation. Wild-type MxA, but not MxA(E645R), was able to bind to LACV nucleocapsid protein in coimmunoprecipitation assays, demonstrating the importance of the carboxy-terminal effector domain of MxA. These results illustrate an efficient mechanism of IFN action whereby an essential virus component is trapped in cytoplasmic inclusions and becomes unavailable for the generation of new virus particles.

L5 ANSWER 8 OF 39 MEDLINE on STN

2001556241. PubMed ID: 11601923. Identification and analysis of a new hepadnavirus in white storks. Pult I; Netter H J; Bruns M; Prassolov A; Sirma H; **Hohenberg H**; Chang S F; Frolich K; Krone O; Kaleta E F; Will H. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie an der Universitat Hamburg, Martinistrasse 52, 20251 Hamburg, Germany.) Virology, (2001 Oct 10) 289 (1) 114-28. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB We identified, cloned, and functionally characterized a new avian hepadnavirus infecting storks (STHBV). STHBV has the largest DNA genome of all avian hepadnaviruses and, based on sequence and phylogenetic analysis, is most closely related to, but distinct from, heron hepatitis B virus (HHBV). Unique for STHBV among the other avian hepadnaviruses is a potential HNF1 binding site in the preS promoter. In common only with HHBV, STHBV has a myristylation signal on the S and not the preS protein, two C terminally located glycosylation sites on the precore/core proteins and lacks the phosphorylation site essential for the transcriptional transactivation activity of duck-HBV preS protein. The cloned STHBV genomes were competent in gene expression, replication, and viral particle secretion. STHBV infected primary duck hepatocytes very inefficiently suggesting a restricted host range, similar to other hepadnaviruses. This discovery of stork infections unravels novel evolutionary aspects of hepadnaviruses and provides new opportunities for hepadnavirus research.

L5 ANSWER 9 OF 39 MEDLINE on STN
2001464920. PubMed ID: 11509913. The human stratum corneum layer: an effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide. Pflucker F; Wendel V; **Hohenberg H**; Gartner E; Will T; Pfeiffer S; Wepf R; Gers-Barlag H. (Research and Development, Merck KGaA, Darmstadt, Germany.) Skin pharmacology and applied skin physiology, (2001) 14 Suppl 1 92-7. Journal code: 9807277. ISSN: 1422-2868. Pub. country: Switzerland. Language: English.
AB Electron microscopy visualisation and light microscopic investigations of three different application forms of titanium dioxide proved that neither surface characteristics, particle size nor shape of the micronised titanium dioxide result in any dermal absorption of this substance: Micronised titanium dioxide is solely deposited on the outermost surface of the stratum corneum and cannot be detected in deeper stratum corneum layers, the human epidermis and dermis.

L5 ANSWER 10 OF 39 MEDLINE on STN
2000469788. PubMed ID: 10888684. Entry of the two infectious forms of vaccinia virus at the plasma membane is signaling-dependent for the IMV but not the EEV. Locker J K; Kuehn A; Schleich S; Rutter G; **Hohenberg H**; Wepf R; Griffiths G. (European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany.. Krijnse@EMBL-Heidelberg.DE) . Molecular biology of the cell, (2000 Jul) 11 (7) 2497-511. Journal code: 9201390. ISSN: 1059-1524. Pub. country: United States. Language: English.
AB The simpler of the two infectious forms of vaccinia virus, the intracellular mature virus (IMV) is known to infect cells less efficiently than the extracellular enveloped virus (EEV), which is surrounded by an additional, TGN-derived membrane. We show here that when the IMV binds HeLa cells, it activates a signaling cascade that is regulated by the GTPase rac1 and rhoA, ezrin, and both tyrosine and protein kinase C phosphorylation. These cascades are linked to the formation of actin and ezrin containing protrusions at the plasma membrane that seem to be essential for the entry of IMV cores. The identical cores of the EEV also appear to enter at the cell surface, but surprisingly, without the need for signaling and actin/membrane rearrangements. Thus, in addition to its known role in wrapping the IMV and the formation of intracellular actin comets, the membrane of the EEV seems to have evolved the capacity to enter cells silently, without a need for signaling.

L5 ANSWER 11 OF 39 MEDLINE on STN
2000445123. PubMed ID: 10994169. [Tissue sampling in the deep head-neck area with a new ultrasound-controlled, semi-automatic micro-punch biopsy device]. Gewebeentnahmen aus dem tiefen Kopf-Hals-Bereich mit einer neuen sonographiegesteuerten, halbautomatischen Mikrostanze. Grundmann T; **Hohenberg H**; Herbst H. (Universitats Hals-Nasen-Ohrenklinik, Hamburg-Eppendorf.) HNO, (2000 Aug) 48 (8) 583-8. Journal code: 2985099R. ISSN: 0017-6192. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.
AB Fine needle aspiration biopsy (FNAB) under ultrasound control is an established diagnostic procedure for the head and neck region. Because of the disintegration of tissues, the diagnostic value of the method is limited resulting in only moderate specificity. In a prospective study, we performed a new, semi-automatic biopsy method in patients who had been diagnosed with sonographically confirmed pathologic masses in the head-neck region. This biopsy is carried out with a spring-loaded biopsy pistol which uses a disposable 20-gauge, specially designed cutting needle. Because this method combines the low invasiveness of FNAB with the high specificity of an excisional biopsy, a high tissue quality is obtained. Comparing these bioptic results with those of subsequent excisional biopsies proves that this new method yields a sensitivity of close to 100% for the detection of lymph node metastases of squamous cell carcinomas (SCC). The tissue cylinders have a reproducible size and allow ultrastructural investigations in the transmission electron microscope

(TEM), on the ultrastructural level. Due to the excellent tissue preservation in the biopsy cylinders, ultrastructural studies, using transmission electron microscopy, may be carried out with the biopsy material. Furthermore, following paraffin embedding of biopsy cylinders, serial sections may be obtained for special staining techniques, and immunohistological investigations are possible which may serve as an adjunct in the diagnosis of, e.g., lymphoproliferative lesions with a sensitivity of 96%. Summarizing, the new semi-automatic biopsy technique obtains tissue probes of high quality with low invasiveness which enables highly sensitive diagnosis of head and neck lesions.

L5 ANSWER 12 OF 39 MEDLINE on STN

2000094903. PubMed ID: 10627527. Biochemical and structural analysis of isolated mature cores of human immunodeficiency virus type 1. Welker R; **Hohenberg H**; Tessmer U; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, D-20251 Hamburg, Germany.) Journal of virology, (2000 Feb) 74 (3) 1168-77. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mature human immunodeficiency virus type 1 (HIV-1) particles contain a cone-shaped core structure consisting of the internal ribonucleoprotein complex encased in a proteinaceous shell derived from the viral capsid protein. Because of their very low stability after membrane removal, HIV-1 cores have not been purified in quantities sufficient for structural and biochemical analysis. Based on our in vitro assembly experiments, we have developed a novel method for isolation of intact mature HIV-1 cores. Concentrated virus suspensions were briefly treated with nonionic detergent and immediately centrifuged in a microcentrifuge for short periods of time. The resuspended pellet was subsequently analyzed by negative-stain and thin-section electron microscopy and by immunoelectron microscopy. Abundant cone-shaped cores as well as tubular and aberrant structures were observed. Stereo images showed that core structures preserved their three-dimensional architecture and exhibited a regular substructure. Detailed analysis of 155 cores revealed an average length of ca. 103 nm, an average diameter at the base of ca. 52 nm, and an average angle of 21.3 degrees. There was significant variability in all parameters, indicating that HIV cores are not homogeneous. Immunoblot analysis of core preparations allowed semiquantitative estimation of the relative amounts of viral and cellular proteins inside the HIV-1 core, yielding a model for the topology of various proteins inside the virion.

L5 ANSWER 13 OF 39 MEDLINE on STN

2000086825. PubMed ID: 10619849. A conformational switch controlling HIV-1 morphogenesis. Gross I; **Hohenberg H**; Wilk T; Wiegers K; Grattinger M; Muller B; Fuller S; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, Martinistrasse 52, D-20251 Hamburg, Germany.) EMBO journal, (2000 Jan 4) 19 (1) 103-13. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Assembly of infectious human immunodeficiency virus type 1 (HIV-1) proceeds in two steps. Initially, an immature virus with a spherical capsid shell consisting of uncleaved Gag polyproteins is formed. Extracellular proteolytic maturation causes rearrangement of the inner virion structure, leading to the conical capsid of the infectious virus. Using an in vitro assembly system, we show that the same HIV-1 Gag-derived protein can form spherical particles, virtually indistinguishable from immature HIV-1 capsids, as well as tubular or conical particles, resembling the mature core. The assembly phenotype could be correlated with differential binding of the protein to monoclonal antibodies recognizing epitopes in the HIV-1 capsid protein (CA), suggesting distinct conformations of this domain. Only tubular and conical particles were observed when the protein lacked spacer peptide SP1 at the C-terminus of CA, indicating that SP1 may act as a molecular switch, whose presence determines spherical capsid formation, while its cleavage leads to maturation.

L5 ANSWER 14 OF 39 MEDLINE on STN

1999424504. PubMed ID: 10496185. Primary structure of the antigen-binding domains of a human oligodendrocyte-reactive IgM monoclonal antibody derived from a patient with multiple sclerosis. Kirschning E; Jensen K; Dubel S; Rutter G; **Hohenberg H**; Will H. (Heinrich-Pette-Institute for Experimental Virology and Immunology at the University of Hamburg, Germany.. kirschni@uke.uni-hamburg.de) . Journal of neuroimmunology, (1999 Sep 1) 99 (1) 122-30. Journal code: 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB Several murine IgM monoclonal antibodies (mAbs) promoting remyelination in mice were shown to be germline gene-encoded natural autoantibodies that react with oligodendrocytes and intracellular antigens. Here, we show that human oligodendrocyte-reactive IgM mAb DS1F8 derived from a patient with multiple sclerosis targets microtubule-like structures similar to the murine mAbs. Sequencing of the cDNAs of the variable regions revealed that the antigen-binding domains are also encoded by germline genes. These similarities of mAb DS1F8 to the murine mAbs promoting remyelination suggest that this human mAb is a natural autoantibody. This may imply that the engineering of human autoantibodies for therapy of demyelinating diseases is feasible.

L5 ANSWER 15 OF 39 MEDLINE on STN

1999225664. PubMed ID: 10208938. In vitro assembly properties of wild-type and cyclophilin-binding defective human immunodeficiency virus capsid proteins in the presence and absence of cyclophilin A. Grattinger M; **Hohenberg H**; Thomas D; Wilk T; Muller B; Krausslich H G. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie an der, Universitat Hamburg, Hamburg, D-20251, Germany.) Virology, (1999 Apr 25) 257 (1) 247-60. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The cellular protein cyclophilin A (CypA) binds specifically to the human immunodeficiency virus type 1 (HIV-1) capsid (CA) protein and is incorporated into HIV-1 particles at a molar ratio of 1:10 (CypA/CA). Structural analysis of a CA-CypA complex suggested that CypA may destabilize interactions in the viral capsid and thus promote uncoating. We analyzed the influence of CypA on the in vitro assembly properties of wild-type (WT) CA and derivatives containing substitutions of Gly89 in the Cyp-binding loop. All variant proteins were significantly impaired in CypA binding. In the presence of CypA at a molar ratio of 1:10 (CypA/CA), WT CA assembled into hollow cylinders that were similar to those observed in the absence of CypA but slightly longer. Higher CypA concentrations inhibited cylinder formation. Variant CA proteins G89L and G89F yielded similar cylinders as the WT protein but were significantly more resistant to CypA. Cryoelectron microscopic analysis of WT cylinders assembled in the presence of CypA revealed direct binding of CypA to the outer surface. Electron diffraction patterns generated from these cylinders indicated that CypA causes local disorder. The addition of CypA to preassembled cylinders had little effect, however, and cylinders were only disrupted when incubated with a threefold molar excess of CypA for several hours. These results suggest that CypA does not efficiently destabilize CA interactions at the molar ratio observed in the virion and therefore is unlikely to serve as an uncoating factor.

L5 ANSWER 16 OF 39 MEDLINE on STN

1998381563. PubMed ID: 9705943. 5000-year-old myelin: uniquely intact in molecular configuration and fine structure. Hess M W; Kirschning E; Pfaller K; Debbage P L; **Hohenberg H**; Klima G. Current biology : CB, (1998 Jul 16) 8 (15) R512-3. Journal code: 9107782. ISSN: 0960-9822. Pub. country: ENGLAND: United Kingdom. Language: English.

L5 ANSWER 17 OF 39 MEDLINE on STN

1998374040. PubMed ID: 9710266. High-pressure freezing and freeze-substitution of native rat brain: suitability for preservation and immunoelectron microscopic localization of myelin glycolipids. Kirschning E; Rutter G; **Hohenberg H**. (Heinrich-Pette-Institute for Experimental

Virology and Immunology at the University of Hamburg, Germany.. Kirschi@hpi.uni-hamburg.de) . Journal of neuroscience research, (1998 Aug 15) 53 (4) 465-74. Journal code: 7600111. ISSN: 0360-4012. Pub. country: United States. Language: English.

AB Galactocerebroside (GalC) and sulfatide are major constituent lipids in vertebrate myelin. Their precise immunolocalization in electron microscopy so far has been hampered by the fact that lipids are not immobilized by chemical fixation and thus get extracted during dehydration with organic solvents. Here, we examined the suitability of cryotechniques for the preservation and immunohistochemical localization of myelin glycolipids in rat brain at the ultrastructural level. Native cerebral cortex tissue, obtained by fine-needle biopsy, was cryoimmobilized by high-pressure freezing and dehydrated by freeze-substitution before embedding in Epon. This procedure resulted in an excellent preservation of brain ultrastructure. Concomitantly, immunogold labeling of ultrathin sections with the well-defined monoclonal antibodies (mAbs) O1, O4, and R-mAb, which were shown to react with GalC and/or sulfatide and some structurally related glycolipids, revealed a good conservation of relevant epitopes. These data suggest that in adult rat cerebral cortex, the most relevant antigens recognized by R-mAb, O1, and O4, namely GalC and sulfatide, are exclusively expressed in myelin structures. Because these mAbs are common markers for the identification of developing oligodendrocytes, this "postembedding glycolipid-labeling technique" holds great potential for studying oligodendroglial differentiation in normal and pathological conditions at the ultrastructural level.

L5 ANSWER 18 OF 39 MEDLINE on STN

1998355278. PubMed ID: 9692550. Cytoplasmic retention of mutant tsp53 is dependent on an intermediate filament protein (vimentin) scaffold. Klotzsche O; Etzrodt D; **Hohenberg H**; Bohn W; Deppert W. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie an der Universitat Hamburg, Germany.) Oncogene, (1998 Jul 2) 16 (26) 3423-34. Journal code: 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The temperature-sensitive mutant tsp53val135 accumulates in the cytoplasm of cells kept at the non-permissive temperature (39 degrees C), but is rapidly transported into the cell nucleus at the permissive temperature (30 degrees C). tsp53 thus may serve as a model for analysing cellular parameters influencing the subcellular location of p53. Here we provide evidence that retention of tsp53 in the cytoplasm at the non-permissive temperature is due to cytoskeletal anchorage of the p53 protein. Two sublines of C6 rat glioma cells differing in their expression of the intermediate filament protein vimentin (vimentin expressing or vimentin negative cells) were stably transfected with a vector encoding tsp53. Whereas cells of vimentin expressing C6 subclones retained tsp53 in the cytoplasm at the non-permissive temperature, cells of vimentin negative subclones exclusively harbored the tsp53 within their nuclei. Intermediate filament deficient cells that had been reconstituted with a full length vimentin protein again showed a cytoplasmic localization of tsp53, whereas in cells expressing a C-terminally truncated (tail-less) vimentin tsp53 localized to the nucleus. We conclude that cytoplasmic sequestration of tsp53 requires an intact intermediate filament system.

L5 ANSWER 19 OF 39 MEDLINE on STN

1998241716. PubMed ID: 9573245. N-Terminal extension of human immunodeficiency virus capsid protein converts the in vitro assembly phenotype from tubular to spherical particles. Gross I; **Hohenberg H**; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut, D-20251 Hamburg, Germany.) Journal of virology, (1998 Jun) 72 (6) 4798-810. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Expression of retroviral Gag polyproteins is sufficient for morphogenesis of virus-like particles with a spherical immature protein shell. Proteolytic cleavage of Gag into the matrix (MA), capsid (CA), nucleocapsid (NC), and p6 domains (in the case of human immunodeficiency virus [HIV]) leads to condensation to the mature cone-shaped core. We

have analyzed the formation of spherical or cylindrical particles on in vitro assembly of purified HIV proteins or inside Escherichia coli cells. CA protein alone yielded cylindrical particles, while all N-terminal extensions of CA abolished cylinder formation. Spherical particles with heterogeneous diameters or amorphous protein aggregates were observed instead. Extending CA by 5 amino acids was sufficient to convert the assembly phenotype to spherical particles. Sequences C-terminal of CA were not required for sphere formation. Proteolytic cleavage of N-terminally extended CA proteins prior to in vitro assembly led to the formation of cylindrical particles, while proteolysis of in vitro assembly products caused disruption of spheres but not formation of cylinders. In vitro assembly of CA and extended CA proteins in the presence of cyclophilin A (CypA) at a CA-to-CypA molar ratio of 10:1 yielded significantly longer cylinders and heterogeneous spheres, while higher concentrations of CypA completely disrupted particle formation. We conclude that the spherical shape of immature HIV particles is determined by the presence of an N-terminal extension on the CA domain and that core condensation during virion maturation requires the liberation of the N terminus of CA.

L5 ANSWER 20 OF 39 MEDLINE on STN

1998184516. PubMed ID: 9525604. Sequential steps in human immunodeficiency virus particle maturation revealed by alterations of individual Gag polyprotein cleavage sites. Wiegers K; Rutter G; Kottler H; Tessmer U; **Hohenberg H**; Krausslich H G. (Heinrich-Pette-Institut, Hamburg, Germany.) Journal of virology, (1998 Apr) 72 (4) 2846-54. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Retroviruses are produced as immature particles containing structural polyproteins, which are subsequently cleaved by the viral proteinase (PR). Extracellular maturation leads to condensation of the spherical core to a capsid shell formed by the capsid (CA) protein, which encases the genomic RNA complexed with nucleocapsid (NC) proteins. CA and NC are separated by a short spacer peptide (spacer peptide 1 [SP1]) on the human immunodeficiency virus type 1 (HIV-1) Gag polyprotein and released by sequential PR-mediated cleavages. To assess the role of individual cleavages in maturation, we constructed point mutations abolishing cleavage at these sites, either alone or in combination. When all three sites between CA and NC were mutated, immature particles containing stable CA-NC were observed, with no apparent effect on other cleavages. Delayed maturation with irregular morphology of the ribonucleoprotein core was observed when cleavage of SP1 from NC was prevented. Blocking the release of SP1 from CA, on the other hand, yielded normal condensation of the ribonucleoprotein core but prevented capsid condensation. A thin, electron-dense layer near the viral membrane was observed in this case, and mutant capsids were significantly less stable against detergent treatment than wild-type HIV-1. We suggest that HIV maturation is a sequential process controlled by the rate of cleavage at individual sites. Initial rapid cleavage at the C terminus of SP1 releases the RNA-binding NC protein and leads to condensation of the ribonucleoprotein core. Subsequently, CA is separated from the membrane by cleavage between the matrix protein and CA, and late release of SP1 from CA is required for capsid condensation.

L5 ANSWER 21 OF 39 MEDLINE on STN

1998036138. PubMed ID: 9370371. In vitro assembly properties of purified bacterially expressed capsid proteins of human immunodeficiency virus. Gross I; **Hohenberg H**; Krausslich H G. (Heinrich-Pette-Institut, Hamburg, Germany.) European journal of biochemistry / FEBS, (1997 Oct 15) 249 (2) 592-600. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The Gag polyprotein of retroviruses is sufficient for assembly and budding of virus-like particles from the host cell. In the case of human immunodeficiency virus (HIV), Gag contains the domains matrix, capsid (CA), nucleocapsid (NC) and p6 which are separated by the viral proteinase inside the nascent virion, leading to morphological maturation to yield an infectious virus. In the mature virus, CA forms a capsid shell

surrounding the ribonucleoprotein core containing CA and the genomic RNA. To define requirements for particle assembly and functional contributions of individual domains, we expressed domains of HIV Gag in Escherichia coli and purified the products to near homogeneity. In vitro assembly of CA, with or without the C-terminally adjacent spacer peptide, yielded tubular structures with a diameter of approximately 55 nm and heterogeneous length. Efficient particle formation required high protein concentration, high salt and neutral to alkaline pH. In contrast, in vitro assembly of CA-NC occurred at a 20-fold lower protein concentration and in low salt, but required addition of RNA. These results suggest that hydrophobic interactions of capsid proteins are sufficient for particle formation while the RNA-binding nucleocapsid domain may concentrate and align structural proteins on the viral genome.

L5 ANSWER 22 OF 39 MEDLINE on STN

97330236. PubMed ID: 9186695. A sulfatide-reactive monoclonal antibody derived from a patient with multiple sclerosis binds to myelin in situ. Kirschning E; Rutter G; Huckhagel C; Ellhof I; **Hohenberg H**. (Heinrich-Pette-Institut fur Experimentelle, Virologie und Immunologie, Universitat Hamburg, Germany.) Annals of the New York Academy of Sciences, (1997 Apr 5) 815 455-8. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

L5 ANSWER 23 OF 39 MEDLINE on STN

96399255. PubMed ID: 8805825. High-pressure freezing of tissue obtained by fine-needle biopsy. **Hohenberg H**; Tobler M; Muller M. (Laboratory for Electron Microscopy I, ETH Zurich, Switzerland.) Journal of microscopy, (1996 Aug) 183 (Pt 2) 133-9. Journal code: 0204522. ISSN: 0022-2720. Pub. country: ENGLAND: United Kingdom. Language: English.

AB High-pressure freezing (HPF) permits adequate cryoimmobilization (without detectable ice crystals after freeze-substitution) of biological tissue up to a thickness of about 200 microns. Until now the preparation of tissue prior to freezing has been unsatisfactory: sizing of the tissue to the required dimensions takes minutes, during which structural alterations must occur. We demonstrate that the use of a fine-needle biopsy technique minimizes tissue damage and guarantees sample dimensions close to the optimal thickness for HPF. The tissue cores can be cryoimmobilized within 40 s of excision.

L5 ANSWER 24 OF 39 MEDLINE on STN

96249402. PubMed ID: 8666745. Detection and spatial distribution of IL-2 receptors on mouse T-lymphocytes by immunogold-labeled ligands. Breitfeld O; Kuhlcke K; Lother H; **Hohenberg H**; Mannweiler K; Rutter G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, Germany.) journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, (1996 Jun) 44 (6) 605-13. Journal code: 9815334. ISSN: 0022-1554. Pub. country: United States. Language: English.

AB To identify the plasma membrane (PM) structures implicated in T-cell activation, we studied the distribution of interleukin-2 receptors (IL-2R) and the surface topography of lymphocytes by affinity labeling in electron microscopy (EM). In particular, we analyzed the distribution of the IL-2R alpha-chain on CTLL-2 cells (a murine cytotoxic T-cell lymphoma line). Some of our experiments were extended to the functionally and morphologically distinct cell line EL4 (a routine helper T-cell lymphoma line). As affinity ligands we used a rat monoclonal antibody (clone 7D4) reactive with the routine alpha-chain of IL-2R and recombinant mouse IL-2 (rIL-2). The distribution of IL-2R was visualized on the cell surface by ligands coupled to colloidal gold particles of different sizes. Unfixed cells were labeled with gold probes and attached to concanavalin A (ConA)-pretreated coverslips. Subsequently, the cells were prepared for EM. Examination of ultrathin sections and large surface replicas revealed a high degree of variability in cell morphology and in the density of the randomly distributed gold-labeled ligands among CTLL cells. According to their typical appearance, lymphocytes with strong receptor expression can be easily identified within the cell population. In contrast, the label

on many mitogen activated EL4 cells showed a cap like polar distribution. The results suggest the existence of diverse distribution patterns of IL-2R on CTLL and EL4 cells. These differences are believed to reflect the different physiological roles played by T-cell subsets in the immune system.

L5 ANSWER 25 OF 39 MEDLINE on STN

95401168. PubMed ID: 7545536. Immuno-gold-labelling of CUT-1, CUT-2 and cuticlin epitopes in Caenorhabditis elegans and Heterorhabditis sp. processed by high pressure freezing and freeze-substitution. Favre R; Hermann R; Cermola M; **Hohenberg H**; Muller M; Bazzicalupo P. (International Institute of Genetics and Biophysics, CNR, Naples, Italy.) Journal of submicroscopic cytology and pathology, (1995 Jul) 27 (3) 341-7. Journal code: 8804312. ISSN: 1122-9497. Pub. country: Italy. Language: English.

AB CUT-1 and CUT-2 are two distinct protein components of cuticlin, the insoluble residue of the cuticles of nematodes. In previous experiments of gold-immuno-labelling on sections of chemically fixed Caenorhabditis elegans, CUT-1 and CUT-2 epitopes were specifically lost. Cryo-immobilization of C. elegans under high pressure followed by freeze-substitution, however, resulted in a good preservation of these antigenic sites and of the ultrastructure of the worms. The entomopathogenic nematode Heterorhabditis sp. processed by the same cryopreparation protocol has shown a strong reactivity with anti-sera raised against CUT-1, CUT-2 and against the whole cuticlin residue of C. elegans. The localization of these epitopes was conserved across the two species.

L5 ANSWER 26 OF 39 MEDLINE on STN

95018184. PubMed ID: 7932676. High-pressure freezing of cell suspensions in cellulose capillary tubes. **Hohenberg H**; Mannweiler K; Muller M. (Heinrich-Pette-Institute for Experimental Virology and Immunology, University of Hamburg, Germany.) Journal of microscopy, (1994 Jul) 175 (Pt 1) 34-43. Journal code: 0204522. ISSN: 0022-2720. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A procedure for efficient cryoimmobilization of large volumes of cell suspensions or micro-organisms by high-pressure freezing is described. This procedure uses transparent, porous cellulose capillary tubes with an inner diameter of 200 microns, into which the suspensions are drawn by capillary action. The tubes are processed by high-pressure freezing and freeze-substitution as if they were tissue samples. Centrifugation of suspensions at low temperatures is no longer necessary and cryopreparation is greatly facilitated. A very high yield of adequately frozen specimens is obtained due to the constant, defined sample geometry. This approach can also be used to process suspensions by conventional chemical fixation, eliminating the need to embed pellets in low-melting-point agarose, for example, prior to chemical fixation. The preparation procedure is demonstrated with suspensions of nematodes, paramecia and bacteria.

L5 ANSWER 27 OF 39 MEDLINE on STN

94307606. PubMed ID: 8034208. Radiation therapy in proliferative vitreoretinopathy. A prospective randomized study. Binder S; Bonnet M; Velikay M; Gerard J P; Stolba U; Wedrich A; **Hohenberg H**. (I. University Eye Hospital Vienna, Austria.) Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie, (1994 Apr) 232 (4) 211-4. Journal code: 8205248. ISSN: 0721-832X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB In a prospective study of the effect of postoperative radiation therapy for the prevention of reproliferation of membranes and recurrent proliferative vitreoretinopathy (PVR) two similar groups of patients with retinal detachment and PVR grade D1 to D3 in one eye were compared. Half the eyes (30) received a total dose of 3000 cGy after surgery; the other half remained untreated. After a follow-up of 6 months and 14 months or more (maximum 36 months) the anatomical and functional results of each group were compared. After 6 months in the unirradiated group 57% (17/30)

remained attached and 10% (3/30) had detached again. In the irradiated group 63% (19/30) were attached and 37% (11/30) had detached. However, there was no statistically significant difference between the two groups (P = 0.479, Fisher's Exact Test). After 14 months the number of cured and uncured eyes remained the same in the unirradiated group, while in four of the eyes in the irradiated group a later onset of reproliferation and detachment occurred (after 7, 8, 12 and 14 months, respectively). A final cure rate of 57% (17/30) was achieved in the unirradiated group and a 50% (15/30) cure rate in the irradiated group. Thus the failure rate was 43% (13/30) in the unirradiated group and 50% (15/30) in the irradiated group (P = 0.473, Fisher's Exact Test). No side effects from the radiation were observed in any case and no radiation retinopathy occurred during an observation period of up to 3 years.(ABSTRACT TRUNCATED AT 250 WORDS)

L5 ANSWER 28 OF 39 MEDLINE on STN

94216371. PubMed ID: 8163549. Characterization of polymer release from the flagellar pocket of Leishmania mexicana promastigotes. Stierhof Y D; Ilg T; Russell D G; **Hohenberg H**; Overath P. (Max-Planck-Institute fur Biologie, Tubingen, Federal Republic of Germany.) Journal of cell biology, (1994 Apr) 125 (2) 321-31. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Trypanosomatids contain a unique compartment, the flagellar pocket, formed by an invagination of the plasma membrane at the base of the flagellum, which is considered to be the sole cellular site for endocytosis and exocytosis of macromolecules. The culture supernatant of Leishmania mexicana promastigotes, the insect stage of this protozoan parasite, contains two types of polymers: a filamentous acid phosphatase (sAP) composed of a 100-kD phosphoglycoprotein with non-covalently associated proteo high molecular weight phosphoglycan (proteo-HMWPG) and fibrous material termed network consisting of complex phosphoglycans. Secretion of both polymers is investigated using mAbs and a combination of light and electron microscopic techniques. Long filaments of sAP are detectable in the lumen of the flagellar pocket. Both sAP filaments and network material emerge from the ostium of the flagellar pocket. While sAP filaments detach from the cells, the fibrous network frequently remains associated with the anterior end of the parasites and can be found in the center of cell aggregates. The related species L. major forms similar networks. Since polymeric structures cannot be detected in intracellular compartments, it is proposed that monomeric or, possibly, oligomeric subunits synthesized in the cells are secreted into the flagellar pocket. Polymer formation from subunits is suggested to occur in the lumen of the pocket before release into the culture medium or, naturally, into the gut of infected sandflies.

L5 ANSWER 29 OF 39 MEDLINE on STN

94072298. PubMed ID: 8251263. Cytoskeleton architecture of C6 rat glioma cell subclones differing in intermediate filament protein expression. Bohn W; Roser K; **Hohenberg H**; Mannweiler K; Traub P. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, Germany.) Journal of structural biology, (1993 Jul-Aug) 111 (1) 48-58. Journal code: 9011206. ISSN: 1047-8477. Pub. country: United States. Language: English.

AB Whole-mount electron microscopy was used in conjunction with immunogold labeling to characterize the cytoskeleton architecture of C6 rat glioma cell subclones. These subclones differ in intermediate filament (IF) protein composition and either contain vimentin (subclone C6D8) or do not express any of the known cytoplasmic IF proteins (subclone C6D10) (Roser et al., 1991). In C6D8 cells short thin (3 nm) connecting filaments frequently linked vimentin to actin filaments and, in addition, connected vimentin filaments to each other. Occasionally, direct contacts were noticed between actin and vimentin filaments. Thin connecting filaments were present at a significantly higher number in IF-deficient C6D10 cells, forming a dense cytoplasmic network in conjunction with actin filament bundles as the dominating structure. The data indicate that thin connecting filaments are present in C6 cells independent of the expression of cytoplasmic IF proteins. They suggest that structural linkages between

play a major role in determining the cytoskeleton architecture of these cells.

L5 ANSWER 30 OF 39 MEDLINE on STN

93294219. PubMed ID: 8515050. Label-fracture of plasma membranes isolated from measles virus-infected cells. Rutter G; **Hohenberg H.** (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, Germany.) journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, (1993 Jul) 41 (7) 1085-91. Journal code: 9815334. ISSN: 0022-1554. Pub. country: United States. Language: English.

AB We present a method that permits correlation of the intramembrane architecture of plasma membrane fracture faces with the distribution of specific molecules at the corresponding cytoplasmic or exoplasmic membrane surfaces. HeLa cells infected with measles virus were used as a model system. Large fragments of the dorsal membrane were isolated after the virus glycoproteins were tagged at the outer cell surface with immune serum and protein A-gold markers. In a second step, different virus polypeptides at the inner cell surface were also identified by a smaller gold label. Thereafter, the isolated plasma membranes were frozen and freeze-fractured. The complementary fracture faces were shadowed with heavy metals and carbon and examined in the transmission electron microscope without cleaning of remaining biological material. Thus, the micromorphology of the replicated fracture faces and the topochemistry of virus components localized at the corresponding leaflets of the plasmalemma could be seen on the same image at high resolution. Of note is that the freeze-fracture morphology of the protoplasmic face is related to the molecular composition of the cytoplasmic surface, as revealed by antibody tagging.

L5 ANSWER 31 OF 39 MEDLINE on STN

91073137. PubMed ID: 2174964. Immunoelectron microscopy on the topographical distribution of the poliovirus receptor. Mannweiler K; Nobis P; **Hohenberg H;** Bohn W. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, F.R.G.) Journal of general virology, (1990 Nov) 71 (Pt 11) 2737-40. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The topographical distribution of the poliovirus receptor on the cell surface was demonstrated by immunoelectron microscopy using monoclonal antibodies and immunogold markers. The receptor appeared in small clusters, which were randomly distributed over the cell surface and along cellular processes. The distribution pattern of the clusters corresponded to that of absorbed and immunogold-labelled poliovirus particles and suggests a multivalent organization of poliovirus binding sites. Freeze-fracturing and ultrathin sectioning did not reveal any specific ultrastructures within the plasma membrane at labelled receptor areas. Incubation of native cells with anti-receptor antibodies did not remove the receptor molecule from the cell surface nor did it induce ultrastructural alterations within the plasma membrane. The antibody-receptor complexes exhibited lateral mobility within the plasma membrane and were able to aggregate into large immune complexes after incubation with a second ligand.

L5 ANSWER 32 OF 39 MEDLINE on STN

88273997. PubMed ID: 3292642. Demonstration of antigens at both sides of plasma membranes in one coincident electron microscopic image: a double-immunogold replica study of virus-infected cells. Rutter G; Bohn W; **Hohenberg H;** Mannweiler K. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie, Universitat Hamburg, Federal Republic of Germany.) journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, (1988 Aug) 36 (8) 1015-21. Journal code: 9815334. ISSN: 0022-1554. Pub. country: United States. Language: English.

AB We present here a procedure for obtaining high-resolution topographical information about the spatial distribution of antigens at both sides of isolated plasma membranes. HeLa cells grown on coverslips and infected

with measles virus served as a model system. Virus glycoproteins appearing at the cell surface were demonstrated by tagging them with rabbit anti-measles antibodies and protein A-gold probes. Cells were stabilized with tannic acid, covered with a cationized coverslip, and then split in potassium-containing buffer. Membranes adherent to the cationized coverslip were fixed in formaldehyde-glutaraldehyde and reacted with mouse monoclonal antibodies against various structural proteins of measles virus. Antibody binding sites at the cytoplasmic surface were visualized either by the antibody bridge method, using normal mouse Ig coupled to gold colloid of different sizes, or by the peroxidase-antiperoxidase procedure. After osmication and critical point-drying, the cytoplasmic surfaces were replicated by platinum-carbon evaporation and examined by TEM without prior cleaning from biological material. This new method permits concomitant localization of antigens present at the inner and outer leaflets of the plasma membrane, and provides high-resolution information about the three-dimensional organization of the cytoplasmic surface.

L5 ANSWER 33 OF 39 MEDLINE on STN

87234204. PubMed ID: 3109026. Replica-immunogold technique applied to studies on measles virus morphogenesis. Bohn W; Mannweiler K; **Hohenberg H**; Rutter G. Scanning microscopy, (1987 Mar) 1 (1) 319-30. Journal code: 8704616. ISSN: 0891-7035. Pub. country: United States. Language: English.

AB The replica technique was applied to studies on the dynamic process of measles virus budding on infected HeLa cells. Virus structures were identified by labeling with anti-measles antibodies and protein A-gold. The combination of these two methods enabled us to characterize the sequence of virus budding at the plasma membrane, to localize virus structures on cytoskeletons of infected cells, and to study the influence of Ca2+ ions on virus structures at the plasma membrane. Studies on platinum carbon surface replicas suggest that the process of virus budding is similar to the genesis of cellular microvilli. Replicas prepared from cytoskeletons of infected cells reveal a close association of budding virus with actin filaments composing the outer parts of the networks. Replicas of apical plasma membranes isolated from infected cells show the attachment of viral nucleocapsids to the protoplasmic membrane face of infected cells. These nucleocapsids are not present on membranes prepared from cells treated with calcium and the ionophore A23187. In addition viral cell surface antigens become randomly distributed on these cells. The data suggest that measles virus morphogenesis at the plasma membrane of cultured cells is dependent on the function of the cytoskeleton and may be influenced by Ca2+ ions.

L5 ANSWER 34 OF 39 MEDLINE on STN

86164378. PubMed ID: 3956518. Preparation of apical plasma membranes from cells grown on coverslips. Electron microscopic investigations of the protoplasmic surface. Rutter G; Bohn W; **Hohenberg H**; Mannweiler K. European journal of cell biology, (1986 Jan) 39 (2) 443-8. Journal code: 7906240. ISSN: 0171-9335. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB We introduce here a simple method which permits an efficient isolation of apical plasma membranes from tissue culture cells and the electron microscopic examination of their protoplasmic surfaces by use of the platinum/carbon replica technique. Different procedures were tested with regard to the efficiency of isolation and preservation of ultrastructure. Best results were obtained by prestabilization of cell surfaces with low concentrations of tannic acid prior to isolation. To demonstrate the possible applications and versatility of the method, studies were done on virus-infected cells in combination with immunocytochemical labeling. With this model system, we show that it is possible to correlate the structures seen on the cytoplasmic surface of the plasma membrane with the distribution of virus antigens at the cell surface labeled with immunogold markers prior to preparation.

L5 ANSWER 35 OF 39 MEDLINE on STN

86124712. PubMed ID: 3946081. Involvement of actin filaments in budding of

measles virus: studies on cytoskeletons of infected cells. Bohn W; Rutter G; **Hohenberg H**; Mannweiler K; Nobis P. Virology, (1986 Feb) 149 (1) 91-106. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Cytoskeletons were prepared from measles virus infected HeLa cells to investigate the involvement of cytoskeletal filaments in virus budding at the plasma membrane. The cytoskeletons retained nearly 80% of measles virus hemagglutinin, the major viral polypeptides, including P, NP, and M, and 2 to 12% of the total cell bound infectivity. As demonstrated with platinum- and carbon-shadowed cytoskeletons, all stages of budding, i.e., virus specific strands, stub-like protrusions, and completely rounded virus particles, are associated with actin filaments composing the outer part of the cytoskeletal network. As shown with ultrathin sections of flat embedded extracted cells, actin filaments identified with heavy meromyosin almost exclusively protrude into virus particles with their barbed ends and are in close association with viral nucleocapsids. The data support previous suggestions that actin is involved in virus budding and show that budding itself is possibly the result of a vectorial growth of actin filaments.

L5 ANSWER 36 OF 39 MEDLINE on STN

84046712. PubMed ID: 6138898. Inhibition of measles virus budding by phenothiazines. Bohn W; Rutter G; **Hohenberg H**; Mannweiler K. Virology, (1983 Oct 15) 130 (1) 44-55. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB HeLa cells infected with measles virus show an accumulation of virus-specific strands at the plasma membrane after addition of the anticalmodulin drugs trifluoperazine (TFP) and chlorpromazine (CPZ), whereas spherical virus particles are almost completely absent. At low drug concentrations (10-15 microM TFP; 30-40 microM CPZ) the inhibitory effect is dependent on the presence of extracellular calcium. The strands complete the budding process after removal of the drugs. Restoration of virus budding is not sensitive to cycloheximide and immunoprecipitation experiments give evidence that the viral protein synthesis is not qualitatively altered in the presence of TFP. The data indicate that both drugs arrest the budding process at an intermediate stage at the plasma membrane. The inability of the strands to comigrate with cytochalasin B-induced actin patches suggests that the inhibition of budding is probably the result of an impaired interaction of viral structures with the cytoskeleton.

L5 ANSWER 37 OF 39 MEDLINE on STN

82216786. PubMed ID: 7086887. Protein-A gold particles as markers in replica immunocytochemistry: high resolution electron microscope investigations of plasma membrane surfaces. Mannweiler K; **Hohenberg H**; Bohn W; Rutter G. Journal of microscopy, (1982 May) 126 (Pt 2) 145-9. Journal code: 0204522. ISSN: 0022-2720. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Due to their high atomic number contrast in transmission electron microscopy, gold particles are ideal markers in surface replicas of cultured cells. The suitability of protein-A-coated gold particles in replica immunocytochemistry for labelling surface antigens is demonstrated using measles virus-infected cells as a model system. Labelled areas can easily be distinguished from unlabelled areas, and even markers positioned in the evaporation shadow of large structures can be accurately identified, which is a prerequisite for an exact quantification and mapping of antigen. In addition, the ultrastructure of labelled areas can still be visualized because of the small size of the marker.

L5 ANSWER 38 OF 39 MEDLINE on STN

81099289. PubMed ID: 7005707. Semi-automatic washing device for simultaneous cleaning of surface replicas under identical conditions. **Hohenberg H**; Mannweiler K. Mikroskopie, (1980 Sep) 36 (5-6) 145-54. Journal code: 0376654. ISSN: 0026-3702. Pub. country: Austria. Language: English.

80143528. PubMed ID: 395456. The galloylglucose mordanting effect as postfixative for tissue culture cells during SEM studies. Mannweiler K; Baigent C L; Rutter G; Andresen I; Neumayer U; **Hohenberg H**. Mikroskopie, (1979 Aug) 35 (5-6) 127-32. Journal code: 0376654. ISSN: 0026-3702. Pub. country: Austria. Language: English.

```
=> d his

     (FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

     FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
               E PREVELIGE P/AU

     FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1             1 S US5716613/PN
L2             1 S US5789245/PN

     FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
               E PREVELIGE P E/AU
L3            35 S E3 OR E4 OR E5
               E KLISHKO V Y/AU
L4             4 S E2-E4
               E GROSS I/AU
               E HOHENBERG H/AU
L5            39 S E3 OR E4

=> e campbell s/au
E1            12      CAMPBELL RUTH/AU
E2             3      CAMPBELL RUTH C/AU
E3           864 --> CAMPBELL S/AU
E4            17      CAMPBELL S A/AU
E5            54      CAMPBELL S B/AU
E6            58      CAMPBELL S C/AU
E7            55      CAMPBELL S D/AU
E8            76      CAMPBELL S E/AU
E9            30      CAMPBELL S F/AU
E10           33      CAMPBELL S G/AU
E11           10      CAMPBELL S H/AU
E12           26      CAMPBELL S J/AU

=> s e3
L6           864 "CAMPBELL S"/AU

=> e rein a/au
E1             2      REIMUS PAUL W/AU
E2             1      REIMUTH NIELS/AU
E3            97 --> REIN A/AU
E4             1      REIN A G/AU
E5            61      REIN A J/AU
E6             3      REIN A J J T/AU
E7             3      REIN A L/AU
E8            11      REIN ALAN/AU
E9             1      REIN ALISON L/AU
E10            4      REIN AZARIA J J T/AU
E11            5      REIN B/AU
E12            3      REIN B E/AU

=> s e3
L7            97 "REIN A"/AU

=> s l6 and l7
L8             4 L6 AND L7

=> d l8,ti,1-4
```

L8 ANSWER 1 OF 4 MEDLINE on STN
TI Modulation of HIV-like particle assembly in vitro by inositol phosphates.

L8 ANSWER 2 OF 4 MEDLINE on STN
TI Infectivity of Moloney murine leukemia virus defective in late assembly events is restored by late assembly domains of other retroviruses.

L8 ANSWER 3 OF 4 MEDLINE on STN
TI The human immunodeficiency virus type 1 Gag polyprotein has nucleic acid chaperone activity: possible role in dimerization of genomic RNA and placement of tRNA on the primer binding site.

L8 ANSWER 4 OF 4 MEDLINE on STN
TI In vitro assembly properties of human immunodeficiency virus type 1 Gag protein lacking the p6 domain.

=> d l8,cbib,ab,1-4

L8 ANSWER 1 OF 4 MEDLINE on STN
2001504162. PubMed ID: 11526217. Modulation of HIV-like particle assembly in vitro by inositol phosphates. **Campbell S**; Fisher R J; Towler E M; Fox S; Issaq H J; Wolfe T; Phillips L R; **Rein A**. (HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702-1201, USA.. cambells@mail.ncifcrf.gov) . Proceedings of the National Academy of Sciences of the United States of America, (2001 Sep 11) 98 (19) 10875-9. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB HIV-1 Gag protein assembles into 100- to 120-nm diameter particles in mammalian cells. Recombinant HIV-1 Gag protein assembles in a fully defined system in vitro into particles that are only 25-30 nm in diameter and that differ significantly in other respects from authentic particles. However, particles with the size and other properties of authentic virions were obtained in vitro by addition of inositol phosphates or phosphatidylinsitol phosphates to the assembly system. Thus, the interactions between HIV-1 Gag protein molecules are altered by binding of inositol derivatives; this binding is apparently essential for normal HIV-1 particle assembly. This requirement is not seen in a deleted Gag protein lacking residues 16-99 within the matrix domain.

L8 ANSWER 2 OF 4 MEDLINE on STN
2000405838. PubMed ID: 10906179. Infectivity of Moloney murine leukemia virus defective in late assembly events is restored by late assembly domains of other retroviruses. Yuan B; **Campbell S**; Bacharach E; **Rein A**; Goff S P. (Integrated Program in Cellular, Molecular and Biophysical Studies, College of Physicians and Surgeons, Columbia University, New York, New York 10032, USA.) Journal of virology, (2000 Aug) 74 (16) 7250-60. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The p12 region of the Moloney murine leukemia virus (M-MuLV) Gag protein contains a PPPY motif important for efficient virion assembly and release. To probe the function of the PPPY motif, a series of insertions of homologous and heterologous motifs from other retroviruses were introduced at various positions in a mutant gag gene lacking the PPPY motif. The assembly defects of the PPPY deletion mutant could be rescued by insertion of a wild-type PPPY motif and flanking sequences at several ectopic positions in the Gag protein. The late assembly domain (L-domain) of Rous sarcoma virus (RSV) or human immunodeficiency virus type 1 (HIV-1) could also fully or partially restore M-MuLV assembly when introduced into matrix, p12, or nucleocapsid domains of the mutant M-MuLV Gag protein lacking the PPPY motif. Strikingly, mutant viruses carrying the RSV or the HIV-1 L-domain at the original location of the deleted PPPY motif were replication competent in rodent cells. These data suggest that the PPPY motif of M-MuLV acts in a partially position-independent manner and is functionally interchangeable with L-domains of other retroviruses.

electron microscopy studies revealed that deletion of the entire p6 region resulted in the formation of tube-like rather than spherical particles. Remarkably, the PPPY deletion mutant formed chain structures composed of multiple viral particles linked on the cell surface. Many of the mutants with heterologous L-domains released virions with wild-type morphology.

L8 ANSWER 3 OF 4 MEDLINE on STN

1999214364. PubMed ID: 10196321. The human immunodeficiency virus type 1 Gag polyprotein has nucleic acid chaperone activity: possible role in dimerization of genomic RNA and placement of tRNA on the primer binding site. Feng Y X; **Campbell S**; Harvin D; Ehresmann B; Ehresmann C; **Rein A**. (Retroviral Genetics Section, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702, USA.) Journal of virology, (1999 May) 73 (5) 4251-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The formation of an infectious retrovirus particle requires several RNA-RNA interaction events. In particular, the genomic RNA molecules form a dimeric structure, and a cellular tRNA molecule is annealed to an 18-base complementary region (the primer binding site, or PBS) on the genomic RNA, where it will serve as primer for reverse transcription. tRNAs normally possess a highly stable secondary and tertiary structure; it seems unlikely that annealing of a tRNA molecule to the PBS, which involves unwinding of this structure, could occur efficiently at physiological temperatures without the assistance of a cofactor. Many prior studies have shown that the viral nucleocapsid (NC) protein can act as a nucleic acid chaperone (i.e., facilitate annealing events between nucleic acids), and the assays used to demonstrate this activity include its ability to catalyze dimerization of transcripts representing retroviral genomes and the annealing of tRNA to the PBS in vitro. However, mature NC is not required for these events in vivo, since protease-deficient viral mutants, in which NC is not cleaved from the parental Gag polyprotein, are known to contain dimeric RNAs with tRNA annealed to the PBS. In the present experiments, we have tested recombinant human immunodeficiency virus type 1 Gag polyprotein for nucleic acid chaperone activity. The protein was positive by all of our assays, including the ability to stimulate dimerization and to anneal tRNA to the PBS in vitro. In quantitative experiments, its activity was approximately equivalent on a molar basis to that of NC. Based on these results, we suggest that the Gag polyprotein (presumably by its NC domain) catalyzes the annealing of tRNA to the PBS during (or before) retrovirus assembly in vivo.

L8 ANSWER 4 OF 4 MEDLINE on STN

1999139010. PubMed ID: 9971810. In vitro assembly properties of human immunodeficiency virus type 1 Gag protein lacking the p6 domain. **Campbell S; Rein A.** (ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702, USA.. campbells@mail.ncifcrf.gov) . Journal of virology, (1999 Mar) 73 (3) 2270-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Human immunodeficiency virus type 1 (HIV-1) normally assembles into particles of 100 to 120 nm in diameter by budding through the plasma membrane of the cell. The Gag polyprotein is the only viral protein that is required for the formation of these particles. We have used an in vitro assembly system to examine the assembly properties of purified, recombinant HIV-1 Gag protein and of Gag missing the C-terminal p6 domain (Gag Deltap6). This system was used previously to show that the CA-NC fragment of HIV-1 Gag assembled into cylindrical particles. We now report that both HIV-1 Gag and Gag Deltap6 assemble into small, 25- to 30-nm-diameter spherical particles in vitro. The multimerization of Gag Deltap6 into units larger than dimers and the formation of spherical particles required nucleic acid. Removal of the nucleic acid with NaCl or nucleases resulted in the disruption of the multimerized complexes. We conclude from these results that (i) N-terminal extension of HIV-1 CA-NC

than cylindrical, particles; (ii) nucleic acid is required for the assembly and maintenance of HIV-1 Gag Deltap6 virus-like particles in vitro and possibly in vivo; (iii) a wide variety of RNAs or even short DNA oligonucleotides will support assembly; (iv) protein-protein interactions within the particle must be relatively weak; and (v) recombinant HIV-1 Gag Deltap6 and nucleic acid are not sufficient for the formation of normal-sized particles.

```
=> d his

     (FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

     FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
               E PREVELIGE P/AU

     FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1             1 S US5716613/PN
L2             1 S US5789245/PN

     FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
               E PREVELIGE P E/AU
L3            35 S E3 OR E4 OR E5
               E KLISHKO V Y/AU
L4             4 S E2-E4
               E GROSS I/AU
               E HOHENBERG H/AU
L5            39 S E3 OR E4
               E CAMPBELL S/AU
L6           864 S E3
               E REIN A/AU
L7            97 S E3
L8             4 S L6 AND L7

=> e ehrlich l s/au
E1             3     EHRLICH L I/AU
E2             2     EHRLICH L R/AU
E3            13 --> EHRLICH L S/AU
E4             7     EHRLICH L W/AU
E5             1     EHRLICH LAUREN/AU
E6             2     EHRLICH LAUREN I RICHIE/AU
E7             1     EHRLICH LAUREN R/AU
E8             1     EHRLICH LISA E/AU
E9             1     EHRLICH LORI A/AU
E10          178     EHRLICH M/AU
E11            3     EHRLICH M A/AU
E12            1     EHRLICH M AVRUM/AU

=> s e3
L9            13 "EHRLICH L S"/AU

=> d l9,cbib,ab,1-13

L9   ANSWER 1 OF 13     MEDLINE on STN
2003379850.    PubMed ID: 12915533.   Tsg101 control of human immunodeficiency
     virus type 1 Gag trafficking and release. Goff A; Ehrlich L S; Cohen S
     N; Carter C A. (Department of Molecular Genetics and Microbiology, State
     University of New York at Stony Brook, Stony Brook, New York 11794-5222,
     USA. ) Journal of virology, (2003 Sep) 77 (17) 9173-82.  Journal code:
     0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB   The structural precursor polyprotein of human immunodeficiency virus type
     1, Pr55(gag), contains a proline-rich motif (PTAP) called the "late
     domain" in its C-terminal p6 region that directs release of mature
     virus-like particles (VLPs) from the plasma membranes of gag-transfected
     COS-1 cells.  The motif binds Tsg101 (vacuolar protein-sorting protein 23,
```

accumulation of the wild-type (wt) Gag precursor in a fraction of COS-1 cytoplasm enriched in multivesicular bodies and small particulate components of the plasma membrane (P100) is p6 dependent. Cleavage intermediates and mature CA mainly partitioned with more rapidly sedimenting larger material enriched in components of lysosomes and early endosomes (P27), and this also was p6 dependent. Expression of truncated or full-length Tsg101 proteins interfered with VLP assembly and Gag accumulation in the P100 fraction. This correlated with reduced accumulation of Gag tagged with green fluorescent protein (Gag-GFP) at the plasma membrane and colocalization with the tagged Tsg101 in perinuclear early endosomes, as visualized by confocal microscopy. Fractionation analysis and confocal examination both indicated that the N-terminal region of Tsg101, which contains binding sites for PTAP and ubiquitin (Ub), was required for Gag trafficking to the plasma membrane. Expression of FLAG-tagged Tsg101 with a deletion in the Ub-binding pocket inhibited VLP release almost completely and to a significantly greater extent than expression of the wt tagged Tsg101 protein or Tsg101-FLAG containing a deletion in the PTAP-binding region. The results demonstrate that Gag associates with endosomal trafficking compartments and indicate that efficient release of virus particles from the plasma membrane requires both the PTAP- and Ub-binding functions of Tsg101 to recruit the cellular machinery required for budding.

L9 ANSWER 2 OF 13 MEDLINE on STN

2001361665. PubMed ID: 11423440. HIV-1 capsid protein forms spherical (immature-like) and tubular (mature-like) particles in vitro: structure switching by pH-induced conformational changes. **Ehrlich L S**; Liu T; Scarlata S; Chu B; Carter C A. (Department of Molecular Genetics & Microbiology, State University of New York at Stony Brook, New York 11794, USA.) Biophysical journal, (2001 Jul) 81 (1) 586-94. Journal code: 0370626. ISSN: 0006-3495. Pub. country: United States. Language: English.

AB The viral genome and replicative enzymes of the human immunodeficiency virus are encased in a shell consisting of assembled mature capsid protein (CA). The core shell is a stable, effective protective barrier, but is also poised for dissolution on cue to allow transmission of the viral genome into its new host. In this study, static light scattering (SLS) and dynamic light scattering (DLS) were used to examine the entire range of the CA protein response to an environmental cue (pH). The CA protein assembled tubular structures as previously reported but also was capable of assembling spheres, depending on the pH of the protein solution. The switch from formation of one to the other occurred within a very narrow physiological pH range (i.e., pH 7.0 to pH 6.8). Below this range, only dimers were detected. Above this range, the previously described tubular structures were detected. The ability of the CA protein to form a spherical structure that is detectable by DLS but not by electron microscopy indicates that some assemblages are inherently sensitive to perturbation. The dimers in equilibrium with these assemblages exhibited distinct conformations: Dimers in equilibrium with the spherical form exhibited a compact conformation. Dimers in equilibrium with the rod-like form had an extended conformation. Thus, the CA protein possesses the inherent ability to form metastable structures, the morphology of which is regulated by an environmentally-sensitive molecular switch. Such metastable structures may exist as transient intermediates during the assembly and/or disassembly of the virus core.

L9 ANSWER 3 OF 13 MEDLINE on STN

2001222327. PubMed ID: 11312344. Structural consequences of cyclophilin A binding on maturational refolding in human immunodeficiency virus type 1 capsid protein. Dietrich L; **Ehrlich L S**; LaGrassa T J; Ebbets-Reed D; Carter C. (Department of Molecular Genetics and Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794-5222, USA.) Journal of virology, (2001 May) 75 (10) 4721-33. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB While several cellular proteins are incorporated in the human immunodeficiency virus type 1 virion, cyclophilin (CyP) A is the only one

whose absence has been demonstrated to impair infectivity. Incorporation of the cytosolic protein results from interaction with a highly exposed Pro-rich loop in the N-terminal region of the capsid (CA) domain of the precursor polyprotein, Pr55(Gag). Even when prevented from interacting with CyP A, Pr55Gag still forms particles that proceed to mature into morphologically wild-type virions, suggesting that CyP A influences a postassembly event. The nature of this CyP A influence has yet to be elucidated. Here, we show that while CyP A binds both Gag and mature CA proteins, the two binding interactions are actually different. Tryptophan 121 (W121) in CyP A distinguished the two proteins: a phenylalanine substitution (W121F) impaired binding of mature CA protein but not of Gag. This indicates the occurrence of a maturation-dependent switch in the conformation of the Pro-rich loop. A structural consequence of Gag binding to CyP A was to block this maturational refolding, resulting in a 24-kDa CA protein retaining the immature Pro-rich loop conformation. Using trypsin as a structure probe, we demonstrate that the conformation of the C-terminal region in mature CA is also a product of maturational refolding. Binding to wild-type CyP A altered this conformation, as indicated by a reduction in the accessibility of Cys residue(s) in the region to chemical modification. Hence, the end result of binding to CyP A, whether the Pro-rich loop is in the context of Gag or mature CA protein, is a structurally modified mature CA protein. The postassembly role of CyP A may be mediated through these modified mature CA proteins.

L9 ANSWER 4 OF 13 MEDLINE on STN

1998181018. PubMed ID: 9514761. Membrane-induced alterations in HIV-1 Gag and matrix protein-protein interactions. Scarlata S; **Ehrlich L S**; Carter C A. (Department of Physiology and Biophysics, S.U.N.Y. Stony Brook, NY 11794, USA.) Journal of molecular biology, (1998 Mar 27) 277 (2) 161-9. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The matrix (MA) domain of human immunodeficiency virus type 1 (HIV-1) contains sequences that direct association with the nucleus at early times in the virus replication cycle and with the plasma membrane at late times in the cycle. Localization to these sites is critical for functions related to the establishment of the infecting provirus and viral assembly, respectively. Mutational and structural analyses indicate that the opposing targeting signals which mediate these subcellular localization events include the same basic residues found in the N-terminal region of the protein. Here, we examined protein multimerization as a determinant of membrane association. Under high ionic strength conditions, Gag, but not MA, binds phospholipid membranes with high affinity. The oligomerization state of the protein per se did not appear to be a prerequisite for stable membrane binding, as Gag and MA were both capable of forming oligomers in high ionic strength buffer. To determine the fate of Gag and MA multimers in the presence of phospholipid membranes in real time, we measured resonance energy transfer between oligomer subunits in the presence and absence of lipid. The presence of phospholipid significantly increased the efficiency of resonance energy transfer between Gag molecules, consistent with enhanced Gag multimerization. This suggests that Gag oligomers assembled on the membrane surface and correlated with the observed stability of membrane binding. In contrast, the efficiency of resonance energy transfer between MA molecules decreased, indicating that MA oligomers dissociated in the presence of membrane, consistent with observed unstable binding. Identical results were obtained whether the probes were covalently attached to a Lys residue in Gag or to residues specifically within the MA domain of Gag; whether the fluorophore was rhodamine or fluorescein; or whether hetero- or homotransfer was measured. The results suggest that phospholipid induces alterations in Gag and MA protein-protein interactions that may contribute to the puzzling ability of MA to direct targeting functions requiring alternately membrane binding and membrane dissociation. The results also suggest that regions downstream of the MA domain in the precursor, or conformations formed after maturation of MA, play a critical role in oligomerization-modulated membrane binding.

L9 ANSWER 5 OF 13 MEDLINE on STN

96378787. PubMed ID: 8784350. Crystal structure of dimeric HIV-1 capsid protein. Momany C; Kovari L C; Prongay A J; Keller W; Gitti R K; Lee B M; Gorbalenya A E; Tong L; McClure J; **Ehrlich L S**; Summers M F; Carter C; Rossmann M G. (Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA.) Nature structural biology, (1996 Sep) 3 (9) 763-70. Journal code: 9421566. ISSN: 1072-8368. Pub. country: United States. Language: English.

AB X-ray diffraction analysis of a human immunodeficiency virus (HIV-1) capsid (CA) protein shows that each monomer within the dimer consists of seven alpha-helices, five of which are arranged in a coiled coil-like structure. Sequence assignments were made for two of the helices, and tentative connectivity of the remainder of the protein was confirmed by the recent solution structure of a monomeric N-terminal fragment. The C-terminal third of the protein is mostly disordered in the crystal. The longest helices in the coiled coil-like structure are separated by a long, highly antigenic peptide that includes the binding site of an antibody fragment complexed with CA in the crystal. The site of binding of the Fab, the position of the antigenic loop and the site of cleavage between the matrix protein and CA establish the side of the dimer that would be on the exterior of the retroviral core.

L9 ANSWER 6 OF 13 MEDLINE on STN

96264770. PubMed ID: 8672424. Partitioning of HIV-1 Gag and Gag-related proteins to membranes. **Ehrlich L S**; Fong S; Scarlata S; Zybarth G; Carter C. (Department of Molecular Genetics & Microbiology, State University of New York at Stony Brook, New York, 11794, USA.) Biochemistry, (1996 Apr 2) 35 (13) 3933-43. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The binding of HIV-1 Gag and Gag-related proteins to model membranes was examined using three experimental systems: (i) large unilamellar phospholipid vesicles (LUVs) and recombinant Gag purified from Escherichia coli; (ii) LUVs added to a mammalian cell extract in which Gag proteins were expressed by a coupled transcription/translation system; and (iii) inside-out plasma membrane vesicles purified from human red blood cells (RBC) and recombinant, purified Gag from E. coli. Several novel aspects of HIV-1 Gag membrane interactions were observed: (i) Gag proteins bound with high affinity to both model membranes with a negatively charged surface and to RBC membranes. (ii) Binding of the Gag precursor and mature Gag proteins exhibited different sensitivities to ionic strength indicating that the precursor directed membrane binding through interactions that were qualitatively and quantitatively distinct from those of any of its individual domains. Studies using energy transfer between tryptophan residues in the proteins and anthroyloxy-containing probes inserted in the LUVs indicated that the orientation of the precursor and of the mature proteins on the membrane surface were distinct; (iii) Gag oligomers appear to have facilitated high-affinity binding under high salt conditions, suggesting that protein-protein interactions led to formation of stronger electrostatic or new hydrophobic membrane binding determinants. Since binding studies with model membranes permit quantitative analysis, these experimental approaches may permit identification of interactions that drive Gag assembly on the membrane.

L9 ANSWER 7 OF 13 MEDLINE on STN

96064217. PubMed ID: 8523337. A preliminary evaluation of hydroxyurea for the treatment of rheumatoid arthritis. **Ehrlich L S**; Thalji K; Whitman K; Albert D A. (Section of Rheumatology, University of Chicago, IL 60637, USA.) Journal of rheumatology, (1995 Sep) 22 (9) 1646-50. Journal code: 7501984. ISSN: 0315-162X. Pub. country: Canada. Language: English.

AB OBJECTIVE. To obtain preliminary evidence on the safety and efficacy of low dose hydroxyurea as a treatment for rheumatoid arthritis (RA). METHODS. Five patients with active RA unresponsive to conventional therapy were entered into a 12 week, open label trial of hydroxyurea followed by a one month postdrug evaluation. RESULTS. Three of the 4 patients completing the study had a decrease in morning stiffness and

number of swollen and tender joints. All 4 patients had a decrease in pain and an increase in function as measured by a modified health assessment questionnaire. No patient had improvement in sedimentation rate, C-reactive protein, or subjective measures of global well being. However, 3 of the 4 patients had disease flare after the drug was withdrawn, demonstrated by increased number of swollen and tender joints. CONCLUSION. Low dose hydroxyurea may be effective in the treatment of RA, but confirmation will require further testing by a randomized double blind placebo controlled trial of patients with a broader spectrum of disease severity over a longer period of therapeutic intervention.

L9 ANSWER 8 OF 13 MEDLINE on STN

95027680. PubMed ID: 7941318. Spectral analysis and tryptic susceptibility as probes of HIV-1 capsid protein structure. **Ehrlich L S**; Agresta B E; Gelfand C A; Jentoft J; Carter C A. (Department of Microbiology, S.U.N.Y. at Stony Brook 11794.) Virology, (1994 Nov 1) 204 (2) 515-25. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The structures of HIV-1 capsid protein (CA, p24) isolated from mature virions and CA protein autoprocessed from a recombinant Gag-Pol precursor expressed in Escherichia coli were compared using circular dichroic (CD) spectral analysis. The spectra obtained for the intact recombinant and viral proteins were indistinguishable, indicating that the backbone configurations directed by the primary amino acid sequences of the proteins were similar or identical. The structure predictions derived from CD were, in general, inconsistent with a model proposing the eight-stranded beta barrel motif found in several RNA viruses. However, aspects of the model were supported by experiments that identified surface-exposed regions. Biochemical analysis indicated that the recombinant CA protein formed nonrandom higher-ordered structures in vitro. Under physiological conditions, the protein assembled into oligomers containing subunits in two packing arrangements. In one arrangement, the central region near Arg100 was exposed and susceptible to tryptic digestion at low enzyme concentrations (enzyme:substrate ratios = 1:5000 to 1:100). Also, in this arrangement, the proteins were susceptible to crosslinking by the bifunctional agent DTSSP. Proteins in the other arrangement were resistant to proteolysis at low enzyme concentrations. The central region of these resistant molecules was inaccessible to monospecific antibodies that recognized antigenic sites between residues 94 and 107 and these proteins were not crosslinked by DTSSP or EGS. Following incubation with trypsin, both the resistant molecules and the fragments derived from the susceptible proteins in the oligomer migrated as smaller complexes, suggesting that the regions digested by trypsin stabilized the oligomer unit. The results indicate that the central region of the HIV-1 CA protein plays a role in formation of higher-ordered structures. Moreover, the relative stability of the N- and C-terminal partial digestion fragments arising from cleavage at Arg100/Gly101 suggests that this exposed central region separates two structural domains of the protein.

L9 ANSWER 9 OF 13 MEDLINE on STN

93245875. PubMed ID: 8482334. Transformation of NIH/3T3 to anchorage independence by H-ras is accompanied by loss of suppressor activity. Tolsma S S; Cohen J D; **Ehrlich L S**; Bouck N P. (Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, Illinois 60611.) Experimental cell research, (1993 Apr) 205 (2) 232-9. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB Despite their familiar sensitivity to transformation by dominant-acting ras oncogenes, NIH/3T3 cells carry a ras suppressor. When tested by cell fusion they were able to suppress the anchorage-independent phenotype of both mouse and human cells transformed by activated H-ras or N-ras. This suppression occurred without a decrease in expression of the activated ras oncogene. Ras-transformed NIH/3T3 clones cured of their oncogene by benzamide treatment reverted to a nontransformed phenotype, but had lost the ability to suppress other ras transformants, indicating that their

cells an active ras oncogene increased the rate of chromosome segregation by > 100-fold. These results suggest that in vitro transformation of NIH/3T3 cells by ras may be more similar to multistep in vivo tumor development than previously suspected, involving not only expression of an active oncogene but also loss of a suppressor activity, perhaps induced by the clastogenic oncogene.

L9 ANSWER 10 OF 13 MEDLINE on STN

93144002. PubMed ID: 1283309. Inactivation of the human immunodeficiency virus by hypericin: evidence for photochemical alterations of p24 and a block in uncoating. Degar S; Prince A M; Pascual D; Lavie G; Levin B; Mazur Y; Lavie D; **Ehrlich L S**; Carter C; Meruelo D. (Department of Pathology, New York University Medical Center, NY 10016.) AIDS research and human retroviruses, (1992 Nov) 8 (11) 1929-36. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Following attachment and entry of human immunodeficiency virus (HIV) into a host cell, the HIV genomic RNA is reverse transcribed to cDNA. This step may be inhibited by hypericin, a compound that induces alterations of the retroviral capsid. Incubation of HIV with hypericin rendered the virus noninfectious. The replication of HIV was blocked early; HIV cDNA could not be detected in cells challenged with hypericin-treated HIV. Hypericin did not inhibit the binding of recombinant gp120 to CD4+ cells, nor did hypericin inhibit syncytium formation. However, reverse transcriptase activity could not be released from hypericin-treated virions. Western blot analysis revealed altered mobility of the HIV major capsid protein (p24) following hypericin treatment. Hypericin-treated recombinant HIV p24 exhibited similar altered mobility. The inactivation of HIV infectivity and the alterations in p24 mobility required hypericin incubations in the presence of visible light. Collectively, these data suggest that photochemical alterations of the HIV capsid may contribute to the hypericin-mediated inactivation of HIV. Such alterations may inhibit the release of RT activity from treated HIV, and prevent uncoating and subsequent reverse transcription of the HIV genome within a target cell.

L9 ANSWER 11 OF 13 MEDLINE on STN

92333669. PubMed ID: 1629958. Assembly of recombinant human immunodeficiency virus type 1 capsid protein in vitro. **Ehrlich L S**; Agresta B E; Carter C A. (Department of Microbiology, State University of New York, Stony Brook 11794.) Journal of virology, (1992 Aug) 66 (8) 4874-83. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The capsid protein (CA) (p24) of human immunodeficiency virus (HIV) type 1 expressed in Escherichia coli and purified to greater than 90% homogeneity was used to examine assembly in vitro and to probe the nature of interactions involved in the formation of capsid structures. The protein was detected in dimeric and oligomeric forms as indicated by molecular size measurements by gel filtration column chromatography, sedimentation through sucrose, and nondenaturing gel electrophoresis. Chemical cross-linking of CA molecules was observed with several homobifunctional reagents. Oligomer size was dependent on cross-linker concentration and exhibited a nonrandom pattern in which dimers and tetramers were more abundant than trimers and pentamers. Oligomers as large as dodecamers were detected in native polyacrylamide gels. These were stable in solutions of high ionic strength or in the presence of nonionic detergent, indicating that strong interactions were involved in oligomer stabilization. Limited tryptic digestion converted the putative dodecamers to octamers, suggesting that a region involved in CA protein multimerization was exposed in the structure. This region was mapped to the central portion of the protein. The recombinant CA proteins assembled in vitro into long rodlike structures and were disassembled into small irregular spheres by alterations in ionic strength and pH. The observation that assembly and disassembly of purified HIV type 1 CA protein can be induced in vitro suggests an approach for identifying possible control mechanisms involved in HIV viral core assembly.

91088635. PubMed ID: 2124709. Preparation and crystallization of a human immunodeficiency virus p24-Fab complex. Prongay A J; Smith T J; Rossmann M G; **Ehrlich L S**; Carter C A; McClure J. (Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Dec) 87 (24) 9980-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A recombinant form of human immunodeficiency virus capsid protein, p24, expressed in Escherichia coli has been purified to homogeneity and separated into distinct isoelectric forms. A monoclonal antibody, mAb25.4, which recognizes an epitope in the amino-terminal region of p24, has been purified to homogeneity from ascites fluid and digested with papain to produce the respective antigen-binding fragment (Fab). The Fab25.4 was purified from the digestion mixture and separated into two distinct isoelectric forms. The two Fab species were each complexed with one isoelectric form of the recombinant p24 by incubating equimolar quantities of the two proteins. Two different crystal morphologies of the p24-Fab25.4 complex were obtained by the vapor-diffusion method with 12-24% PEG 3350 as the precipitant. One of these crystal forms has unit-cell parameters of a = 92.1 A, b = 85.4 A, c = 54.0 A, alpha = gamma = 90.0 degrees and beta = 90.4 degrees and belongs to the monoclinic space group P2(1), with one molecule of the complex per asymmetric unit. These crystals strongly diffracted x-rays to at least 2.7-A resolution.

L9 ANSWER 13 OF 13 MEDLINE on STN

91069614. PubMed ID: 2123631. Expression in Escherichia coli and purification of human immunodeficiency virus type 1 capsid protein (p24). **Ehrlich L S**; Krausslich H G; Wimmer E; Carter C A. (Department of Microbiology, State University of New York Stony Brook 11794.) AIDS research and human retroviruses, (1990 Oct) 6 (10) 1169-75. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Capsid protein (p24;CA) of human immunodeficiency virus type 1 (HIV-1) was synthesized in Escherichia coli strain BL21 (DE3) using a plasmid encoding a truncated HIV-1 gag/pol gene. The plasmid, which contained a mutation in the frameshift region, expressed viral proteinase (PR), a pol gene product, in the gag reading frame, resulting in efficient processing of mature CA and other gag-related products. The expressed CA is soluble, recognized by monoclonal antibodies directed against HIV CA and has an N-terminal sequence identical to that of CA purified from HIV. Purification was done under mild conditions where coexpressed HIV PR retained enzymatic activity. Milligram quantities of 90% pure CA protein were obtained after chromatography on DEAE cellulose followed by facilitated aggregation of the CA in the unbound fraction. The precipitated CA was readily dissolved in low ionic strength aqueous buffer. Gel exclusion chromatography results indicated that, in solution, CA existed in oligomeric form.

=> d his

(FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
E PREVELIGE P/AU

FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1 1 S US5716613/PN
L2 1 S US5789245/PN

FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
E PREVELIGE P E/AU
L3 35 S E3 OR E4 OR E5
E KLISHKO V Y/AU
L4 4 S E2-E4
E GROSS I/AU

```
L5            39 S E3 OR E4
                 E CAMPBELL S/AU
L6           864 S E3
                 E REIN A/AU
L7            97 S E3
L8             4 S L6 AND L7
                 E EHRLICH L S/AU
L9            13 S E3

=> e klikova m/au
E1             1     KLIKOVA CHRISTIANA/AU
E2             1     KLIKOVA D/AU
E3             2 --> KLIKOVA M/AU
E4             1     KLIKOWSKA K/AU
E5             1     KLIKS B/AU
E6             1     KLIKS B R/AU
E7             3     KLIKS I/AU
E8             4     KLIKS M/AU
E9            13     KLIKS M M/AU
E10            5     KLIKS S/AU
E11           10     KLIKS S C/AU
E12            1     KLIKSBERG B/AU

=> s e3
L10            2 "KLIKOVA M"/AU

=> d l10,cbib,ab,1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L10  ANSWER 1 OF 2     MEDLINE on STN
97459967.    PubMed ID: 9312040.   The three-dimensional solution structure of
     the matrix protein from the type D retrovirus, the Mason-Pfizer monkey
     virus, and implications for the morphology of retroviral assembly. Conte M
     R; Klikova M; Hunter E; Ruml T; Matthews S. (Department of Biochemistry,
     Imperial College of Science, Technology and Medicine, University of
     London, London SW7 2AY, UK. ) EMBO journal, (1997 Oct 1) 16 (19) 5819-26.
     Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United
     Kingdom. Language: English.
AB   The Mason-Pfizer monkey virus (M-PMV) is the prototype of the type D
     retroviruses.  In type B and D retroviruses, the Gag protein pre-assembles
     before association with the membrane, whereas in type C retroviruses
     (lentiviruses, BLV/HTLV group) Gag is targeted efficiently to the plasma
     membrane, where the particle formation occurs.  The N-terminal domain of
     Gag, the matrix protein (MA), plays a critical role in determining this
     morphogenic difference.  We have determined the three-dimensional solution
     structure of the M-PMV MA by heteronuclear nuclear magnetic resonance.
     The protein contains four alpha-helices that are structurally similar to
     the known type C MA structures.  This similarity implies possible common
     assembly units and membrane-binding mechanisms for type C and B/D
     retroviruses.  In addition to this, the interpretation of mutagenesis data
     has enabled us to identify, for the first time, the structural basis of a
     putative intracellular targeting motif.

L10  ANSWER 2 OF 2     MEDLINE on STN
95115066.    PubMed ID: 7815488.   Efficient in vivo and in vitro assembly of
     retroviral capsids from Gag precursor proteins expressed in bacteria.
     Klikova M; Rhee S S; Hunter E; Ruml T. (Department of Biochemistry and
     Microbiology, Institute of Chemical Technology, Prague, Czech Republic. )
     Journal of virology, (1995 Feb) 69 (2) 1093-8.  Journal code: 0113724.
     ISSN: 0022-538X. Pub. country: United States. Language: English.
AB   The capsid precursor protein (Gag) of Mason-Pfizer monkey virus, the
     prototype type D retrovirus, has been expressed to high levels in bacteria
     under the control of the phage T7 promoter.  Electron microscopic studies
     of induced cells revealed the assembly of capsid-like structures within
     inclusion bodies that formed at the poles of the cells 6 h after induction
```

with isopropyl beta-D-thiogalactopyranoside (IPTG). The inducible system and enclosed capsid-like structures were solubilized completely in 8 M urea, but following renaturation, we observed assembly in vitro of capsid-like structures that demonstrated apparent icosahedral symmetry. These results demonstrate for the first time that retroviral capsid precursors have the propensity to self-assemble in vitro and point to new approaches for the analysis of retroviral assembly and structure.

```
=> e sakalian m/au
E1            2     SAKALAUSKIENE L/AU
E2            5     SAKALES S R/AU
E3            6 --> SAKALIAN M/AU
E4            8     SAKALIAN MICHAEL/AU
E5            4     SAKALIDOU A/AU
E6            1     SAKALIENE JURGITA/AU
E7            2     SAKALIENE ONA/AU
E8           27     SAKALIHASAN N/AU
E9            4     SAKALIHASAN NATZI/AU
E10          10     SAKALIHASSAN N/AU
E11           1     SAKALIKOVA A/AU
E12           9     SAKALINSKAS M A/AU

=> s e3 or e4
             6 "SAKALIAN M"/AU
             8 "SAKALIAN MICHAEL"/AU
L11         14 "SAKALIAN M"/AU OR "SAKALIAN MICHAEL"/AU

=> d l11,cbib,ab,1-14

L11  ANSWER 1 OF 14      MEDLINE on STN
2004284894.     PubMed ID: 15183067.   Isolation and characterization of the
     Mason-Pfizer monkey virus p12 protein. Knejzlik Zdenek; Strohalm Martin;
     Sedlackova Lenka; Kodicek Milan; Sakalian Michael; Ruml Tomas.
     (Department of Biochemistry and Microbiology and Center for Integrated
     Genomics, Institute of Chemical Technology, 166 28 Prague, Czech Republic.
     ) Virology, (2004 Jun 20) 324 (1) 204-12.  Journal code: 0110674. ISSN:
     0042-6822. Pub. country: United States. Language: English.
AB   The Mason-Pfizer monkey virus (M-PMV) Gag protein, precursor to the
     structural proteins of the infectious virion, assembles into immature
     capsid-like particles when expressed at high levels in bacterial cells.
     Similar capsid-like particles can be obtained by in vitro assembly using a
     high concentration of isolated Gag.  M-PMV Gag contains a p12 protein that
     has no corresponding analogues in most other retroviruses and has been
     suggested to contain an internal scaffold domain (ISD).  We have expressed
     and purified p12 and the N- and C-terminal halves (Np12 and Cp12) that are
     predicted to be structurally independent domains.  The behavior of these
     proteins was analyzed using chemical cross-linking, CD spectroscopy, and
     electron microscopy.  The N-terminal half of p12 is largely alpha-helical
     although the C-terminal portion lacks any apparent ordered structure.
     Both p12 and Np12 form high-order oligomers in vitro and when expressed in
     E. coli produce organized structures that are visible by electron
     microscopy.  Interestingly, Cp12, as well as the whole protein, can form
     dimers in the presence of SDS.  The data show that both domains of p12
     contribute to its ability to multimerize with much of this potential
     residing in its N-terminal part, most probably within the leucine
     zipper-like (LZL) sequence.

L11  ANSWER 2 OF 14      MEDLINE on STN
2004050766.     PubMed ID: 14752833.   Cloning the human betaretrovirus proviral
     genome from patients with primary biliary cirrhosis. Xu Lizhe; Sakalian
     Michael; Shen Zhiwei; Loss George; Neuberger James; Mason Andrew.
     (Section of Gastroenterology and Hepatology, Ochsner Clinic Foundation,
     New Orleans, LA, USA. ) Hepatology (Baltimore, Md.), (2004 Jan) 39 (1)
     151-6.  Journal code: 8302946. ISSN: 0270-9139. Pub. country: United
     States. Language: English.
```

patients with primary biliary cirrhosis (PBC), have been serologic and tissue evidence of infection. A recently identified human betaretrovirus was originally cloned from the biliary epithelium cDNA library of a patient with PBC. By conducting a BLASTN search, the initial partial pol gene fragment was found to have 95% to 97% nucleotide homology with mouse mammary tumor virus (MMTV) and with retrovirus sequences derived from human breast cancer samples. Using an anti-p27(CA) MMTV antibody, viral proteins were detected in the perihepatic lymph nodes but not in liver tissue samples from patients with PBC, suggesting a higher viral burden in lymphoid tissue. Therefore, in the current study, we used lymph node DNA to clone the proviral genome of the human betaretrovirus from two patients with PBC using a polymerase chain reaction (PCR) walking methodology with conserved primers complementary to MMTV. The human betaretrovirus genome contains five potential open reading frames (ORF) for Gag, protease (Pro), polymerase (Pol), envelope (Env), and superantigen (Sag) proteins that are collinear with their counterparts in MMTV. Alignment studies performed with characterized MMTV and human breast cancer betaretrovirus amino acid sequences revealed a 93% to 99% identity with the p27 capsid proteins, a 93% to 97% identity with the betaretrovirus envelope proteins, and a 76% to 85% identity with the more variable superantigen proteins. Phylogenetic analysis of known betaretrovirus superantigen proteins showed that the human and murine sequences did not cluster as two distinct species. In conclusion, human betaretrovirus nucleic acid sequences have been cloned from patients with PBC. They share marked homology with MMTV and human breast cancer-derived retrovirus sequences.

L11 ANSWER 3 OF 14 MEDLINE on STN

2003391245. PubMed ID: 12879148. Membrane transplantation to correct integral membrane protein defects. Curlee Kimberly V; Hong Jeong S; Clancy J P; King Scott A; Hunter Eric; Berdiev Bakhrom; Benos Dale; Sommerfelt Maja A; Sorscher Eric J; **Sakalian Michael**. (Department of Human Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, USA.) Journal of molecular medicine (Berlin, Germany), (2003 Aug) 81 (8) 511-20. Journal code: 9504370. ISSN: 0946-2716. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB In this report we show that the tendency of certain viruses to carry host membrane proteins in their envelopes can be harnessed for transplantation of small patches of plasma membrane, including fully functional, polytopic ion channel proteins and their regulatory binding partners. As a stringent model we tested the topologically complex epithelial ion channel CFTR. Initially an attenuated vaccinia virus was found capable of transferring CFTR in a properly folded, functional and regulatable form to CFTR negative cells. Next we generated viruslike particles (VLPs) composed of retroviral structural proteins that assemble and bud at the host cell plasma membrane. These particles were also shown to mediate functional ion channel transfer. By testing the capacity of complex membrane proteins to incorporate into viral envelopes these experiments provide new insight into the permissiveness of viral envelopment, including the ability of incorporated proteins to retain function and repair defects at the cell surface, and serve as a platform for studies of ion channel and membrane protein biochemistry.

L11 ANSWER 4 OF 14 MEDLINE on STN

2003001739. PubMed ID: 12507478. Identification of novel interactions in HIV-1 capsid protein assembly by high-resolution mass spectrometry. Lanman Jason; Lam TuKiet T; Barnes Stephen; **Sakalian Michael**; Emmett Mark R; Marshall Alan G; Prevelige Peter E Jr. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294-2170, USA.) Journal of molecular biology, (2003 Jan 24) 325 (4) 759-72. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB The pleomorphic nature of the immature and mature HIV-1 virions has made it difficult to characterize intersubunit interactions using traditional approaches. While the structures of isolated domains are known, the challenge is to identify intersubunit interactions and thereby pack these domains into supramolecular structures. Using high-resolution mass

spectrometry, we have measured the amide hydrogen exchange protection factors for the soluble capsid protein (CA) and CA assembled in vitro. Comparison of the protection factors as well as chemical crosslinking experiments has led to a map of the subunit/subunit interfaces in the assembled tubes. This analysis provides direct biochemical evidence for the homotypic N domain and C domain interactions proposed from cryo-electron microscopy image reconstruction of CA tubes. Most significantly, we have identified a previously unrecognized intersubunit N domain-C domain interaction. The detection of this interaction reconciles previously discrepant biophysical and genetic data.

L11 ANSWER 5 OF 14 MEDLINE on STN

2002696015. PubMed ID: 12239217. Conserved residues in the putative catalytic triad of human bile acid Coenzyme A:amino acid N-acyltransferase. Sfakianos Mindan K; Wilson Landon; **Sakalian Michael**; Falany Charles N; Barnes Stephen. (Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA.) Journal of biological chemistry, (2002 Dec 6) 277 (49) 47270-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Human bile acid-CoA:amino acid N-acyltransferase (hBAT), an enzyme catalyzing the conjugation of bile acids with the amino acids glycine or taurine has significant sequence homology with dienelactone hydrolases and other alpha/beta hydrolases. These enzymes have a conserved catalytic triad that maps onto the mammalian BATs at residues Cys-235, Asp-328, and His-362 of the human sequence, albeit that the hydrolases contain a serine instead of a cysteine. In the present study, the function of the putative catalytic triad of hBAT was examined by chemical modification with the cysteine alkylating reagent N-ethylmaleimide (NEM) and by site-directed mutagenesis of the triad residues followed by enzymology studies of mutant and wild-type hBATs. Treatment with NEM caused inactivation of wild-type hBAT. However, preincubation of wild-type hBAT with the substrate cholyl-CoA before NEM treatment prevented loss of N-acyltransferase activity. Substitution of His-362 or Asp-328 with alanine results in inactivation of hBAT. Although substitution of Cys-235 with serine generated an hBAT mutant with lower N-acyltransferase activity, it substantially increased the bile acid-CoA thioesterase activity compared with wild type. In summary, data from this study support the existence of an essential catalytic triad within hBAT consisting of Cys-235, His-362, and Asp-328 with Cys-235 serving as the probable nucleophile and thus the site of covalent attachment of the bile acid molecule.

L11 ANSWER 6 OF 14 MEDLINE on STN

2002611458. PubMed ID: 12368324. The Mason-Pfizer monkey virus internal scaffold domain enables in vitro assembly of human immunodeficiency virus type 1 Gag. **Sakalian Michael**; Dittmer Stephanie S; Gandy A Dustin; Rapp Nathan D; Zabransky Ales; Hunter Eric. (Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190, USA.. mike-sakalian@ouhsc.edu) . Journal of virology, (2002 Nov) 76 (21) 10811-20. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The Mason-Pfizer monkey virus (M-PMV) Gag protein possesses the ability to assemble into an immature capsid when synthesized in a reticulocyte lysate translation system. In contrast, the human immunodeficiency virus (HIV) Gag protein is incapable of assembly in parallel assays. To enable the assembly of HIV Gag, we have combined or inserted regions of M-PMV Gag into HIV Gag. By both biochemical and morphological criteria, several of these chimeric Gag molecules are capable of assembly into immature capsid-like structures in this in vitro system. Chimeric species containing large regions of M-PMV Gag fused to HIV Gag sequences failed to assemble, while species consisting of only the M-PMV p12 region, and its internal scaffold domain (ISD), fused to HIV Gag were capable of assembly, albeit at reduced kinetics compared to M-PMV Gag. The ability of the ISD to induce assembly of HIV Gag, which normally assembles at the plasma membrane, suggests a common requirement for a concentrating factor in retrovirus assembly. Despite the dramatic effect of the ISD on chimera

assembly, the function of HIV Gag domains in this process was found to remain essential, since an assembly-defective mutant of HIV CA, M185A, abolished assembly when introduced into the chimera. This continued requirement for HIV Gag domain function in the assembly of chimeric molecules will allow this in vitro system to be used for the analysis of potential inhibitors of HIV immature particle assembly.

L11 ANSWER 7 OF 14 MEDLINE on STN

2002329867. PubMed ID: 12072491. Kinetic analysis of the role of intersubunit interactions in human immunodeficiency virus type 1 capsid protein assembly in vitro. Lanman Jason; Sexton Jennifer; **Sakalian Michael**; Prevelige Peter E Jr. (Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294-2170, USA.) Journal of virology, (2002 Jul) 76 (14) 6900-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) capsid protein (CA) plays a crucial role in both assembly and maturation of the virion. Numerous recent studies have focused on either the soluble form of CA or the polymer end product of in vitro CA assembly. The CA polymer, in particular, has been used to study CA-CA interactions because it is a good model for the CA interactions within the virion core. However, analysis of the process of in vitro CA assembly can yield valuable insights into CA-CA interactions and the mechanism of core assembly. We describe here a method for the analysis of CA assembly kinetics wherein the progress of assembly is monitored by using turbidity. At pH 7.0 the addition of either of the isolated CA domains (i.e., the N or the C domain) to an assembly reaction caused a decrease in the assembly rate by competing for binding to the full-length CA protein. At pH 8.0 the addition of the isolated C domain had a similar inhibitory affect on CA assembly. However, at pH 8.0 the isolated N domain had no affect on the rate of CA assembly but, when mixed with the C domain, it alleviated the C-domain inhibition. These data provide biochemical evidence for a pH-sensitive homotypic N-domain interaction, as well as for an N- and C-domain interaction.

L11 ANSWER 8 OF 14 MEDLINE on STN

2002155189. PubMed ID: 11886273. Identification of a minimal HIV-1 gag domain sufficient for self-association. Zabransky Ales; Hunter Eric; **Sakalian Michael**. (Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190, USA.) Virology, (2002 Mar 1) 294 (1) 141-50. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Gag polyprotein precursors play an essential role in the assembly of the HIV particle by polymerizing into a spherical shell at the plasma membrane. In order to define the domains within Gag responsible for this homotypic interaction, we have coupled the technology of the yeast two-hybrid system with the technology of a gene-based, semirandom library. By this method, we have identified a minimal region of Gag capable of efficient self-interaction. This region consists of the N-terminal portion of the nucleocapsid protein (NC), including the first zinc finger and the previously described interaction, or I, domain. In parallel with this randomized approach, individual HIV Gag domains, and combinations of these domains, were tested for potential homotypic and heterotypic interactions in the yeast two-hybrid system. Consistent with the results from the semirandom library screen, only combinations of species containing NC were strongly interacting.

L11 ANSWER 9 OF 14 MEDLINE on STN

1999412321. PubMed ID: 10482556. Separate assembly and transport domains within the Gag precursor of Mason-Pfizer monkey virus. **Sakalian M**; Hunter E. (Department of Microbiology, The University of Alabama at Birmingham, Birmingham, Alabama 35294, USA.) Journal of virology, (1999 Oct) 73 (10) 8073-82. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mason-Pfizer monkey virus (M-PMV), the prototypical type D retrovirus,

assembles immature capsids within the cytoplasm of the host prior to plasma membrane interaction. Several mutants of M-PMV Gag have been described which display altered transport, assembly, or both. In this report, we describe the use of an in vitro synthesis and assembly system to distinguish between defects in intracellular transport and the process of assembly itself for two previously described gag gene mutants. Matrix domain mutant R55W converts the type D morphogenesis of M-PMV particles into type C and has been hypothesized to alter the transport of Gag, redirecting it to the plasma membrane where assembly subsequently occurs. We show here that R55W can assemble in both the in vitro translation-assembly system and within inclusion bodies in bacteria and thus has retained the capacity to assemble in the cytoplasm. This supports the concept that R55 is located within a domain responsible for the transport of Gag to an intracellular site for assembly. In contrast, deletions within the p12 domain of M-PMV Gag had previously been shown to affect the efficiency of particle formation such that under low-level expression conditions, Gag would fail to assemble. We demonstrate here that the efficiency of assembly in the in vitro system mirrors that seen in cells under expression conditions similar to that of an infection. These results argue that the p12 domain of this D-type retrovirus plays a critical role in the membrane-independent assembly of immature capsids.

L11 ANSWER 10 OF 14 MEDLINE on STN

1998455620. PubMed ID: 9782300. Molecular events in the assembly of retrovirus particles. **Sakalian M;** Hunter E. (Department of Microbiology, University of Alabama at Birmingham, USA.) Advances in experimental medicine and biology, (1998) 440 329-39. Ref: 33. Journal code: 0121103. ISSN: 0065-2598. Pub. country: United States. Language: English.

AB Retrovirus assembly results from the ability of a single gene product, the gag polyprotein precursor, to coalesce into a spherical particle capable of release from the cell. In conjunction with this primary process of capsid formation additional viral gene products such as the replicative enzymes and envelope glycoproteins as well as the genomic RNA are incorporated to form an infectious virus.

L11 ANSWER 11 OF 14 MEDLINE on STN

1998184547. PubMed ID: 9525635. Type D retrovirus capsid assembly and release are active events requiring ATP. Weldon R A Jr; Parker W B; **Sakalian M;** Hunter E. (Department of Microbiology, University of Alabama at Birmingham, 35294, USA.) Journal of virology, (1998 Apr) 72 (4) 3098-106. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mason-Pfizer monkey virus (M-PMV), the prototype type D retrovirus, differs from most other retroviruses by assembling its Gag polyproteins into procapsids in the cytoplasm of infected cells. Once assembled, the procapsids migrate to the plasma membrane, where they acquire their envelope during budding. Because the processes of M-PMV protein transport, procapsid assembly, and budding are temporally and spatially unlinked, we have been able to determine whether cellular proteins play an active role during the different stages of procapsid morphogenesis. We report here that at least two stages of morphogenesis require ATP. Both procapsid assembly and procapsid transport to the plasma membrane were reversibly blocked by treating infected cells with sodium azide and 2-deoxy-D-glucose, which we show rapidly and reversibly depletes cellular ATP pools. Assembly of procapsids in vitro in a cell-free translation/assembly system was inhibited by the addition of nonhydrolyzable ATP analogs, suggesting that ATP hydrolysis and not just ATP binding is required. Since retrovirus Gag polyproteins do not bind or hydrolyze ATP, these results demonstrate that cellular components must play an active role during retrovirus morphogenesis.

L11 ANSWER 12 OF 14 MEDLINE on STN

96211503. PubMed ID: 8648705. Synthesis and assembly of retrovirus Gag precursors into immature capsids in vitro. **Sakalian M;** Parker S D; Weldon R A Jr; Hunter E. (Department of Microbiology, University of Alabama at Birmingham, 35294-2170, USA.) Journal of virology, (1996 Jun)

United States. Language: English.

AB The assembly of retroviral particles is mediated by the product of the gag gene; no other retroviral gene products are necessary for this process. While most retroviruses assemble their capsids at the plasma membrane, viruses of the type D class preassemble immature capsids within the cytoplasm of infected cells. This has allowed us to determine whether immature capsids of the prototypical type D retrovirus, Mason-Pfizer monkey virus (M-PMV), can assemble in a cell-free protein synthesis system. We report here that assembly of M-PMV Gag precursor proteins can occur in this in vitro system. Synthesized particles sediment in isopycnic gradients to the appropriate density and in thin-section electron micrographs have a size and appearance consistent with those of immature retrovirus capsids. The in vitro system described in this report appears to faithfully mimic the process of assembly which occurs in the host cell cytoplasm, since M-PMV gag mutants defective in in vivo assembly also fail to assemble in vitro. Likewise, the Gag precursor proteins of retroviruses that undergo type C morphogenesis, Rous sarcoma virus and human immunodeficiency virus, which do not preassemble capsids in vivo, fail to assemble particles in this system. Additionally, we demonstrate, with the use of anti-Gag antibodies, that this cell-free system can be utilized for analysis in vitro of potential inhibitors of retrovirus assembly.

L11 ANSWER 13 OF 14 MEDLINE on STN

94335114. PubMed ID: 8057473. Efficiency and selectivity of RNA packaging by Rous sarcoma virus Gag deletion mutants. **Sakalian M**; Wills J W; Vogt V M. (Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14850.) Journal of virology, (1994 Sep) 68 (9) 5969-81. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB In all retrovirus systems studied, the leader region of the RNA contains a cis-acting sequence called psi that is required for packaging the viral RNA genome. Since the pol and env genes are dispensable for formation of RNA-containing particles, the gag gene product must have an RNA binding domain(s) capable of recognizing psi. To gain information about which portion(s) of Gag is required for RNA packaging in the avian sarcoma and leukemia virus system, we utilized a series of gag deletion mutants that retain the ability to assemble virus-like particles. COS cells were cotransfected with these mutant DNAs plus a tester DNA containing psi, and incorporation of RNA into particles were measured by RNase protection. The efficiency of packaging was determined by normalization of the amount of psi+ RNA to the amount of Gag protein released in virus-like particles. Specificity of packaging was determined by comparisons of psi+ and psi- RNA in particles and in cells. The results indicate that much of the MA domain, much of the p10 domain, half of the CA domain, and the entire PR domain of Gag are unnecessary for efficient packaging. In addition, none of these deleted regions is needed for specific selection of the psi RNA. Deletions within the NC domain, as expected, reduce or eliminate both the efficiency and the specificity of packaging. Among mutants that retain the ability to package, a deletion within the CA domain (which includes the major homology region) is the least efficient. We also examined particles of the well-known packaging mutant SE21Q1b. The data suggest that the random RNA packaging behavior of this mutant is not due to a specific defect but rather is the result of the cumulative effect of many point mutations throughout the gag gene.

L11 ANSWER 14 OF 14 MEDLINE on STN

92198737. PubMed ID: 1666295. Changes in surface glycoconjugates in adhesion-defective variants of P19 embryonal carcinoma cells. **Sakalian M**; Draber P. (Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague.) International journal of developmental biology, (1991 Dec) 35 (4) 473-9. Journal code: 8917470. ISSN: 0214-6282. Pub. country: Spain. Language: English.

AB Embryonal carcinoma cells defective in their ability to adhere to tissue culture dishes were isolated from mutagenized P19X1 and P19S1801A1 cells.

three independently isolated variants were analyzed for their morphology, surface properties and ability to differentiate in vitro. Two of the mutant cell lines expressed similar amounts of stage-specific embryonic antigens TEC-1, TEC-4 and Thy-1 as parental cells, whereas all three showed significant reduction in the expression of uvomorulin as determined by a direct radioantibody binding assay. Variant cells exhibited a decrease in their ability to aggregate in media with or without CA2+ and were unable to form compact aggregates when cultured for two days in complete culture media. In the presence of retinoic acid variant cells formed aggregates which exhibited significantly lower frequency neuron formation after transfer to tissue culture dishes. The combined data indicate that the adhesion-defective phenotype of P19-derived cells is in part the result of a reduced surface expression of uvomorulin.

```
=> d his

     (FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

     FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
                E PREVELIGE P/AU

     FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1             1 S US5716613/PN
L2             1 S US5789245/PN

     FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
                E PREVELIGE P E/AU
L3            35 S E3 OR E4 OR E5
                E KLISHKO V Y/AU
L4             4 S E2-E4
                E GROSS I/AU
                E HOHENBERG H/AU
L5            39 S E3 OR E4
                E CAMPBELL S/AU
L6           864 S E3
                E REIN A/AU
L7            97 S E3
L8             4 S L6 AND L7
                E EHRLICH L S/AU
L9            13 S E3
                E KLIKOVA M/AU
L10            2 S E3
                E SAKALIAN M/AU
L11           14 S E3 OR E4

=> e smith a j/au
E1           121     SMITH A I/AU
E2             9     SMITH A IAN/AU
E3           594 --> SMITH A J/AU
E4             1     SMITH A J A/AU
E5             2     SMITH A J D/AU
E6             1     SMITH A J F/AU
E7             2     SMITH A J H/AU
E8             4     SMITH A J JR/AU
E9             1     SMITH A J P/AU
E10           62     SMITH A K/AU
E11          506     SMITH A L/AU
E12           11     SMITH A L JR/AU

=> s e3
L12          594 "SMITH A J"/AU

=> s l12 and py=1990
        388048 PY=1990
L13          17 L12 AND PY=1990
```

```
=> s l13 and assembled
         13457 ASSEMBLED
L14          1 L13 AND ASSEMBLED

=> d l14,cbib,ab

L14  ANSWER 1 OF 1      MEDLINE on STN
90244387.     PubMed ID: 1692347.   Human immunodeficiency virus type 1 Pr55gag
      and Pr160gag-pol expressed from a simian virus 40 late replacement vector
      are efficiently processed and assembled into viruslike particles. Smith
      A J; Cho M I; Hammarskjold M L; Rekosh D. (Department of Microbiology,
      State University of New York, Buffalo 14214. ) Journal of virology, (1990
      Jun) 64 (6) 2743-50.  Journal code: 0113724. ISSN: 0022-538X. Pub.
      country: United States. Language: English.
AB    Human immunodeficiency virus type 1 (HIV-1) gag and pol genes were
      expressed by using fragments of the BH10 clone of HIV inserted into a
      simian virus 40 late replacement vector.  An initial construct containing
      the entire coding regions of gag, pol, and vif produced only minute
      amounts of the gag precursor, Pr55gag.  However, high-level expression was
      obtained when an additional sequence from the env gene (the rev-responsive
      element) was inserted 3' of vif in the correct orientation, and rev was
      provided in trans from a second vector.  Western immunoblot analysis of
      transfected cells showed the presence of large amounts of both Pr55gag and
      Pr160gag-pol as well as all of the expected cleavage products.  Electron
      microscopy of thin sections of transfected cells showed a multitude of
      viruslike particles.  Both immature particles in the process of budding
      and particles containing the condensed core characteristic of HIV were
      observed.  Analysis of the released viruslike particles showed the
      presence of active reverse transcriptase.  Sucrose gradient analysis of
      particles produced from [3H]uridine-labeled cells indicated a peak of
      radioactivity which cosedimented with a peak of p24, suggesting that the
      particles contained RNA.


=> e leis j/au
E1            3     LEIS HENRY T/AU
E2            4     LEIS HUGO/AU
E3           65 --> LEIS J/AU
E4            1     LEIS J C/AU
E5            8     LEIS J F/AU
E6            1     LEIS J M/AU
E7           22     LEIS J P/AU
E8            5     LEIS J R/AU
E9            4     LEIS JONATHAN/AU
E10           2     LEIS JOSE/AU
E11           1     LEIS JOSE F/AU
E12           1     LEIS K/AU

=> s e3
L15          65 "LEIS J"/AU

=> s l15 and py=1988
        365732 PY=1988
L16           8 L15 AND PY=1988

=> d l16,ti,1-8

L16  ANSWER 1 OF 8      MEDLINE on STN
TI    Preliminary crystallographic study of a retroviral protease.

L16  ANSWER 2 OF 8      MEDLINE on STN
TI    Conserved cysteine and histidine residues of the avian myeloblastosis
      virus nucleocapsid protein are essential for viral replication but are not
      "zinc-binding fingers".
```

L16 ANSWER 3 OF 8 MEDLINE on STN
TI A retroviral RNA secondary structure required for efficient initiation of reverse transcription.

L16 ANSWER 4 OF 8 MEDLINE on STN
TI Synthetic peptides as substrates and inhibitors of a retroviral protease.

L16 ANSWER 5 OF 8 MEDLINE on STN
TI Properties of avian sarcoma-leukosis virus pp32-related pol-endonucleases produced in Escherichia coli.

L16 ANSWER 6 OF 8 MEDLINE on STN
TI Standardized and simplified nomenclature for proteins common to all retroviruses.

L16 ANSWER 7 OF 8 MEDLINE on STN
TI Site-directed mutagenesis of the avian retrovirus nucleocapsid protein, pp 12. Mutation which affects RNA binding in vitro blocks viral replication.

L16 ANSWER 8 OF 8 MEDLINE on STN
TI Site-directed mutagenesis of the avian retrovirus nucleocapsid protein, pp 12, at Serine 40, the primary site of phosphorylation in vivo.

=> d l16,cbib,ab,6

L16 ANSWER 6 OF 8 MEDLINE on STN
88188269. PubMed ID: 3357211. Standardized and simplified nomenclature for proteins common to all retroviruses. **Leis J**; Baltimore D; Bishop J M; Coffin J; Fleissner E; Goff S P; Oroszlan S; Robinson H; Skalka A M; Temin H M; +. (Department of Biochemistry, Case Western Reserve University Medical School, Cleveland, Ohio 44106.) Journal of virology, **(1988 May)** 62 (5) 1808-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB We propose a revised standardized nomenclature for the proteins common to all retroviruses on the basis of biological function, enzymatic activity, and/or virion location data. (We do not discuss proteins specific for subfamilies or only some retroviruses.)

=> file uspatful
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 54.25 87.74

FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 24 Jun 2004 (20040624/PD)
FILE LAST UPDATED: 24 Jun 2004 (20040624/ED)
HIGHEST GRANTED PATENT NUMBER: US6754908
HIGHEST APPLICATION PUBLICATION NUMBER: US2004123365
CA INDEXING IS CURRENT THROUGH 24 Jun 2004 (20040624/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 24 Jun 2004 (20040624/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Apr 2004
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Apr 2004

>>> USPAT2 is now available. USPATFULL contains full text of the <<<
>>> original, i.e., the earliest published granted patents or <<<
>>> applications. USPAT2 contains full text of the latest US <<<
>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<

```
>>>  records and may be searched in standard search fields, e.g., /PN,  <<<
>>>  /PK, etc.                                                          <<<

>>>  USPATFULL and USPAT2 can be accessed and searched together         <<<
>>>  through the new cluster USPATALL.  Type FILE USPATALL to           <<<
>>>  enter this cluster.                                                <<<
>>>                                                                     <<<
>>>  Use USPATALL when searching terms such as patent assignees,        <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                            <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s (HIV or human immunodeficiency virus)
         31488 HIV
        392203 HUMAN
         18172 IMMUNODEFICIENCY
         74782 VIRUS
         13017 HUMAN IMMUNODEFICIENCY VIRUS
               (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L17      33171 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l17 and (Gag or CA or NC or MA or capsid or matrix or nucleocapsid)
         21758 GAG
        158252 CA
         30339 NC
         84615 MA
          6874 CAPSID
        324061 MATRIX
          1885 NUCLEOCAPSID
L18      23040 L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOCAPS
               ID)

=> s l18 and (in vitro assembly)
        120671 VITRO
        995647 ASSEMBLY
           192 IN VITRO ASSEMBLY
                 (VITRO(W)ASSEMBLY)
L19         66 L18 AND (IN VITRO ASSEMBLY)

=> s l19 and (assembly or morphogenesis or formation or virus-like particle? or particle?)
        995647 ASSEMBLY
          2908 MORPHOGENESIS
        817055 FORMATION
         74782 VIRUS
       2194589 LIKE
        555589 PARTICLE?
           998 VIRUS-LIKE PARTICLE?
                 (VIRUS(W)LIKE(W)PARTICLE?)
        555589 PARTICLE?
L20         66 L19 AND (ASSEMBLY OR MORPHOGENESIS OR FORMATION OR VIRUS-LIKE
               PARTICLE? OR PARTICLE?)

=> s l20 and ay<2001
       3178100 AY<2001
L21         35 L20 AND AY<2001

=> d l21,ti,1-35

L21  ANSWER 1 OF 35  USPATFULL on STN
TI     Immunomodulatory peptides

L21  ANSWER 2 OF 35  USPATFULL on STN
TI     HLA BINDING PEPTIDES AND THEIR USES
```

L21 ANSWER 3 OF 35 USPATFULL on STN
TI TARGET SPECIFIC SCREENS AND THEIR USE FOR DISCOVERING SMALL ORGANIC MOLECULAR PHARMACOPHORES

L21 ANSWER 4 OF 35 USPATFULL on STN
TI Immunomodulatory peptides

L21 ANSWER 5 OF 35 USPATFULL on STN
TI Fusion proteins comprising coiled-coil structures derived of bovine IF1 ATPase inhibitor protein

L21 ANSWER 6 OF 35 USPATFULL on STN
TI HLA BINDING PEPTIDES AND THEIR USES

L21 ANSWER 7 OF 35 USPATFULL on STN
TI HLA BINDING PEPTIDES AND THEIR USES

L21 ANSWER 8 OF 35 USPATFULL on STN
TI Method for producing chimeric polypeptides

L21 ANSWER 9 OF 35 USPATFULL on STN
TI Methods of quantifying viral load in an animal with a ribonuclease resistant RNA preparation

L21 ANSWER 10 OF 35 USPATFULL on STN
TI TARGETED ARTIFICIAL GENE DELIVERY

L21 ANSWER 11 OF 35 USPATFULL on STN
TI Recombinant retroviral vector

L21 ANSWER 12 OF 35 USPATFULL on STN
TI AAV **capsid** vehicles for molecular transfer

L21 ANSWER 13 OF 35 USPATFULL on STN
TI Protein complexes having factor VIII:C activity and production thereof

L21 ANSWER 14 OF 35 USPATFULL on STN
TI Modified small RNA viruses

L21 ANSWER 15 OF 35 USPATFULL on STN
TI Protein complexes having factor VIII:C activity and production thereof

L21 ANSWER 16 OF 35 USPATFULL on STN
TI Ribonuclease resistant RNA preparation and utilization

L21 ANSWER 17 OF 35 USPATFULL on STN
TI AAV **capsid** vehicles for molecular transfer

L21 ANSWER 18 OF 35 USPATFULL on STN
TI Oral immunization with papillomavirus **virus-like particles**

L21 ANSWER 19 OF 35 USPATFULL on STN
TI Recombinant fibrin chains, fibrin and fibrin-homologs

L21 ANSWER 20 OF 35 USPATFULL on STN
TI Protein complexes having Factor VIII:C activity and production thereof

L21 ANSWER 21 OF 35 USPATFULL on STN
TI Methods for making HLA binding peptides and their uses

L21 ANSWER 22 OF 35 USPATFULL on STN
TI Method of treating malignant tumors with thyroxine analogues having no significant hormonal activity

L21 ANSWER 23 OF 35 USPATFULL on STN
TI Target specific screens and their use for discovering small organic

molecular pharmacophores

L21 ANSWER 24 OF 35 USPATFULL on STN
TI Ribonuclease resistant RNA preparation and utilization

L21 ANSWER 25 OF 35 USPATFULL on STN
TI Human protein critical for **HIV** replication

L21 ANSWER 26 OF 35 USPATFULL on STN
TI Biocompatible implant for the expression and in vivo secretion of a therapeutic substance

L21 ANSWER 27 OF 35 USPATFULL on STN
TI Immunomodulatory peptides

L21 ANSWER 28 OF 35 USPATFULL on STN
TI AAV **capsid** vehicles for molecular transfer

L21 ANSWER 29 OF 35 USPATFULL on STN
TI Immunomodulatory peptides

L21 ANSWER 30 OF 35 USPATFULL on STN
TI Protein complexes having factor VIII:C activity and production thereof

L21 ANSWER 31 OF 35 USPATFULL on STN
TI Method of augmenting soft tissue in mammals

L21 ANSWER 32 OF 35 USPATFULL on STN
TI Methods of treating **HIV** infection using antibodies to the U2 small nuclear ribonuclear protein

L21 ANSWER 33 OF 35 USPATFULL on STN
TI DNA molecules, expression vectors and host cells expressing antigenized antibodies

L21 ANSWER 34 OF 35 USPATFULL on STN
TI Protein complexes having Factor VIII:C activity and production thereof

L21 ANSWER 35 OF 35 USPATFULL on STN
TI Antigenized antibodies and genes

=> d l21,cbib,ab,clm,3

L21 ANSWER 3 OF 35 USPATFULL on STN
2003:133972 TARGET SPECIFIC SCREENS AND THEIR USE FOR DISCOVERING SMALL ORGANIC MOLECULAR PHARMACOPHORES.
BLUME, ARTHUR J., MONTCLAIR, NJ, UNITED STATES
US 2003092057 A1 20030515
APPLICATION: US 1999-412831 A1 19991005 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a general process by which recombinantly derived variable domains of antibodies encompassing either or both light and heavy variable regions with or without respective constant regions are engineered and selected for identification of unique surface domains of pharmaceutical targets or parts thereof which regulate target function. The recombinant antibodies are useful as reagents for high volume, rapid screening of occupation of the active surface domains by natural or synthetic entities. This invention is also directed to elucidating the three dimensional conformation of the antibodies, or parts thereof, which bind to the pharmaceutical targets and confers activity. Methods for creating high resolution molecular models which can direct the synthesis of biologically active small organic molecules useful as viable discovery drug leads are also provided.

CLM What is claimed is:

1. A method of identifying a ligand capable of binding to at least one determinant of a biologically active site on a target, which determinant participates in conferring biological activity of said target, the method comprising: a) providing at least one reporter antibody to be used as a reporter of binding of said ligand to the biologically active site, and wherein said antibody is selected from an antibody library of sufficient diversity to possess at least one antibody member capable of binding to at least one determinant in the biologically active site as determined by the ability of said antibody member, either alone or in combination with at least one other ligand, to possess agonist or antagonist activity; b) identifying as potential ligands for activity at the target, those ligands which are capable of competing with the reporter antibody for binding to the target.

2. The method according to claim 1 wherein the reporter antibodies are members of a recombinant library wherein each antibody member (rVab) of the recombinant library comprises at least one variable region selected from the group consisting of VH and VL regions, and optionally comprising a constant domain attached by its amino terminus to the variable region.

3. The method according to claim 2 wherein the rVab unit is displayed on the surface of a carrier.

4. The method according to claim 2 wherein the rVab unit is soluble.

5. The method according to claim 3 wherein the carrier is a bacteria.

6. The method according to claim 3 wherein the carrier is a bacteriophage.

7. The method according to claim 2 wherein a parental VL region comprising at least one CDR is used to derive the VL region of the rVab by deleting, inserting or substituting at least one amino acid within at least one CDR.

8. The method according to claim 2 wherein a parental VH region comprising at least one CDR is used to derive the VH region of the rVab by deleting, inserting or substituting at least one amino acid within at least one CDR.

9. The method according to claim 2 wherein parental VL and VH regions comprising at least one CDR, are used to derive a pair of VL and VH regions of a rVab by deleting, inserting or substituting at least one amino acid within at least one CDR of each variable region.

10. The method according to any one of claim 7, 8 or 9 wherein the crystal structure of the parental V regions used to derive rVab are known.

11. The method according to claim 9 wherein the crystal structure of the parental VH and VL pair used to derive the rVab is known.

12. The method according to claim 2 wherein at least one of the parental V regions used to derive rVab is unmodified.

13. The method according to claim 2 wherein the crystal structure of the rVab is determined after isolation as a rVab which binds to a biologically active site on the target.

14. The method according to claim 2 wherein at least two V regions are modified by deleting, inserting or substituting at least one amino acid in at least one CDR after isolation as rVab which binds to a biologically active site on the target.

15. The method according to claim 1 wherein the target is a polypeptide,

protein, nucleic acid, oligosaccharide or lipid.

16. The method according to claim 1 wherein activity of the target is coupled to an assayable biochemical response at the target which biochemical response functions as a signal of target activation.

17. The method according to claim 16 wherein the biochemical response is detectable as a change in a protein or polypeptide characteristic.

18. The method according to claim 16 wherein the biochemical response is associated with an organometallic moiety, a metal or other nonprotein.

19. The method according to claim 16 wherein the biochemical response is associated with a portion of the bioactive structure.

20. The method according to claim 16 wherein the biochemical response comprises a detectable free radical, fluorescent or chemiluminsecent group, radioactive isotope or involves oligomerization.

21. The method according to claim 16 wherein the biochemical response is phosphorylation and the signal is a change in the phosphorylation state of the target.

22. The method according to claim 17 wherein the signal protein is a G protein and the signal is a change in either the prepense of a G protein regulatory agent or the binding of rVab due to the presence of a G protein regulatory agent.

23. The method according to claim 16 wherein the signal is a change in the binding of rVab to its binding site.

24. The method according to claim 2 wherein the recombinant antibody comprises a single polypeptide chain comprising a VH functionally coupled to a VL to produce a binding site.

25. A method of identifying ligands capable of binding to at least two determinants which together are required for biological activity of a pharmacological target, the method comprising: a) screening and isolating from an rVab library, rVab members comprising at least one VH and VL regions, and optionally comprising a constant domain attached by its amino terminus to the V region, and capable of binding to at least one of the determinants of the pharmacological target; b) making and expressing an rVab-peptide (rVab-PEP) library comprising the isolated rVab members coupled to at least one peptide comprised of a random sequence of amino acids; c) screening the rVab-PEP library for first rVab-Pep members which bind and activate the pharmacological target wherein the rVab component binds to a first determinant of the pharmacological target and the peptide component binds to a second determinant of the pharmacological target; d) screening the rVab-Pep library and identifying a second rVab-pep member capable of actively binding to the pharmacological target, and wherein the rVab component binds to a third determinant of the pharmacological target and the peptide component binds to fourth determinant of the pharmacological target.

26. The method according to claim 25 wherein the rVab component of the second rVab-Pep member competes with the peptide component of the first rVab-Pep member for binding to a determinant on the pharmacological target.

27. The method according to claim 25 wherein the rVab component of the first rVab-Pep member competes with the peptide component of the second rVab-Pep member for binding to a determinant on the pharmacological target.

28. The method according to claim 25 wherein the first determinant of

the pharmacological target is the same as the second determinant, and wherein the second determinant of the pharmacological target is the same as the third determinant.

29. The method according to claim 25 wherein the rVab component used to construct the rVab-Pep has at least one other attribute of an active ligand, besides affinity for the target, and wherein the attribute is selected from selectivity and biological activity.

30. The method according to claim 29 wherein rVabs which bind to determinants of active sites are identified by their ability to competitively or allosterically alter the binding on an endogenous ligand.

31. The method according to claim 25 wherein the active rVab-Pep possess agonist or antagonist activity.

32. The method according to claim 31 wherein activity of the target is coupled to an assayable biochemical response at the target which biochemical response functions as a signal of target activation.

33. The method according to claim 32 wherein the biochemical response is detectable as a change in a protein or polypeptide characteristic.

34. The method according to claim 32 wherein the biochemical response is associated with an organometallic moiety, a metal or other nonprotein.

35. The method according to claim 32 wherein the biochemical response is associated with a portion of the bioactive structure.

36. The method according to claim 32 wherein the biochemical response comprises a detectable free radical, fluorescent or chemiluminsecent group, radioactive isotope or involves oligomerization.

37. The method according to claim 32 wherein the biochemical response is phosphorylation and the signal is a change in the phosphorylation state of the target.

38. The method according to claim 33 wherein the signal protein is a G protein and the signal is a change in either the prepense of a G protein regulatory agent or the binding of rVab due to the presence of a G protein regulatory agent.

39. The method according to claim 32 wherein the signal is a change in the binding of rVab to its binding site.

40. The method according to claims 25 wherein the peptide component of the rVab-Pep members comprising VH and CL regions are expressed attached to either or both of the amino terminus of VH and the carboxy terminus of CL.

41. The method according claim 40 wherein the peptide component is attached to the amino terminus of the VH region.

42. The method according to claim 40 wherein the peptide component is attached to the carboxy terminus of the CL region.

43. The method according to claim 40 wherein two peptides are attached to the rVab component to form rVab-Pep^2.

44. The method according to claim 40 wherein the peptide comprises between about 5 and 50 amino acids.

45. The method according to claim 44 wherein the peptide comprises between about 7 and 25 amino acids.

46. The method according to claim 45 wherein the peptide comprises about 8 amino acids.

47. A reporter of binding of a ligand to a determinant of a pharmacological target, which target requires binding of ligand to at least two determinants of said target to produce a biological response, said reporter comprising an rVab portion of an active rVab-Pep, and wherein said rVab component of said rVab-Pep binds to a first determinant of said target, and the peptide component binds to a second determinant of said target.

48. The reporter of claim 47 wherein the rVab comprises VH and CL regions and the peptide is expressed bound to either or both of the amino terminus of the VH and the carboxy terminus of the CL.

49. The reporter according claim 48 wherein the peptide component is attached to the amino terminus of the VH region.

50. The reporter according to claim 47 wherein the peptide component is attached to the carboxy terminus of the CL region.

51. The method according to claim 47 wherein two peptides are attached to the rVab component to form rVab-Pep^2.

52. The method according to claim 47 wherein the peptide comprises between about 5 and 50 amino acids.

53. The method according to claim 52 wherein the peptide comprises between about 7 and 25 amino acids.

54. The method according to claim 53 wherein the peptide comprises about 8 amino acids.

55. A method of identifying a ligand capable of binding to at least one determinant of a biologically active site on a target, which target requires activation of at least two determinants to express biological activity of said target, the method comprising: a) providing at least one rVab reporter antibody according to claim 47 to be used as a reporter of binding of said ligand to the biologically active site, and wherein said antibody is selected from an antibody library of one antibody member capable of binding to at least one determinant in the biologically active site as determined by the ability of said antibody member, either alone or in combination with at least one other ligand, to possess agonist or antagonist activity; b) identifying as potential ligands for activity at the target, those ligands which are capable of competing with the reporter antibody for binding to the target.

56. The method according to claim 55 wherein multiple ligands are identified which when bound together covalently, are capable of binding to the determinants necessary to cause a bioligical response of the target, the method comprising: a) providing reporter rVab antibodies for each of the determinants for which ligands are to be identified; b) for each of the rVab reporter antibodies, identifying as potential ligands for activity at each of the determinants of the target, those ligands which are capable of competing with each of the rVab reporter antibodies for binding to the target; c) covalently linking the identified ligands so as to form active multivalent ligands capable of activating the pharmacological target.

57. The method according to claim 56 wherein the identified ligands are non-protein organic molecules.

58. The method according to claim 56 wherein the two rVab reporter antibodies are used to identify two ligands which are combined to form the multivalent active ligand.

59. The method according to claim 58 wherein the pharmacological target is a polypeptide receptor.

60. A recombinant rVab antibody library comprising rVab members possessing at least one VL or VH region derived from a parental variable region with at least one CDR which is diversified to form different rVab members by deleting, inserting or substituting at least one amino acid within at least one CDR.

61. The recombinant antibody library according to claim 60 wherein a parental VH region comprising at least one CDR is used to derive the VH region of the rVab members by deleting, inserting or substituting at least one amino acid within at least one CDR.

62. The recombinant antibody library according to claim 60 wherein parental VL and VH regions comprising at least one CDR, are used to derive a pair of VL and VH regions of rVab members by deleting, inserting or substituting at least one amino acid within at least one CDR of each variable region.

63. The recombinant antibody library according to any one of claim 60, 61, or 62 wherein the crystal structure of the parental V regions used to derive rVab members are known.

64. The recombinant antibody library according to claim 60 wherein the crystal structure of the parental VH and VL pair used to derive the rVab members is known.

65. The recombinant antibody library according to claim 60 wherein at least one of the parental V regions used to derive rVab is unmodified.

66. The recombinant antibody library according to claim 60 wherein the CDR regions of a specific antibody are expressed on a plurality of frameworks which provides for variable geometric orientation of the CDR regions.

67. The recombinant antibody library according to claim 60 wherein the rVab members further comprise a peptide sequence covalently bound to the rVab members to form rVab-Pep members.

68. The recombinant antibody library according to claim 67 wherein the peptide component of the rVab-Pep members comprising VH and CL regions are expressed attached to either or both of the amino terminus of VH and the carboxy terminus of CL.

69. The recombinant antibody library according claim 68 wherein the peptide component is attached to the amino terminus of the VH region.

70. The recombinant antibody library according to claim 68 wherein the peptide component is attached to the carboxy terminus of the CL region.

71. The recombinant antibody library according to claim 68 wherein two peptides are attached to the rVab component to form rVab-Pep^2.

72. The recombinant antibody library according to claim 68 wherein the peptide comprises between about 5 and 50 amino acids.

73. The recombinant antibody library according to claim 72 wherein the peptide comprises between about 7 and 25 amino acids.

74. The recombinant antibody library according to claim 73 wherein the peptide comprises about 8 amino acids.

75. A method of providing a model for a ligand capable of binding to a determinant of an active site of a pharmacological target, the method comprising: a) providing at least two rVab identified as binding to an

active surface of a pharmacological target; b) identifying the regions of the rVabs that bind the biologically active site or individual inactive surface determinants of the bioactive structure; c) grouping the rVabs by overlapping structures which bind to common epitopes; d) determining the relative spatial orientation, charge and energetics of the identified binding sites e) determining the molecular structure necessary to bind the target and confer activity.

=> d his

(FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
E PREVELIGE P/AU

FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1 1 S US5716613/PN
L2 1 S US5789245/PN

FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
E PREVELIGE P E/AU
L3 35 S E3 OR E4 OR E5
E KLISHKO V Y/AU
L4 4 S E2-E4
E GROSS I/AU
E HOHENBERG H/AU
L5 39 S E3 OR E4
E CAMPBELL S/AU
L6 864 S E3
E REIN A/AU
L7 97 S E3
L8 4 S L6 AND L7
E EHRLICH L S/AU
L9 13 S E3
E KLIKOVA M/AU
L10 2 S E3
E SAKALIAN M/AU
L11 14 S E3 OR E4
E SMITH A J/AU
L12 594 S E3
L13 17 S L12 AND PY=1990
L14 1 S L13 AND ASSEMBLED
E LEIS J/AU
L15 65 S E3
L16 8 S L15 AND PY=1988

FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
L17 33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L18 23040 S L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOC
L19 66 S L18 AND (IN VITRO ASSEMBLY)
L20 66 S L19 AND (ASSEMBLY OR MORPHOGENESIS OR FORMATION OR VIRUS-LIKE
L21 35 S L20 AND AY<2001

=> s l17 and (gag/clm or CA/clm or MA/clm or NC/clm or capsid/clm or matrix/clm or nucleocapsid
1030 GAG/CLM
13245 CA/CLM
3367 MA/CLM
2746 NC/CLM
474 CAPSID/CLM
76131 MATRIX/CLM
100 NUCLEOCAPSID/CLM
L22 2371 L17 AND (GAG/CLM OR CA/CLM OR MA/CLM OR NC/CLM OR CAPSID/CLM OR MATRIX/CLM OR NUCLEOCAPSID/CLM)

```
=> s l22 and (assembly/clm or particle?/clm or morphogenesis/clm or assembly/clm or formation/c
        330138 ASSEMBLY/CLM
        149200 PARTICLE?/CLM
            63 MORPHOGENESIS/CLM
        330138 ASSEMBLY/CLM
         89581 FORMATION/CLM
L23        664 L22 AND (ASSEMBLY/CLM OR PARTICLE?/CLM OR MORPHOGENESIS/CLM OR
               ASSEMBLY/CLM OR FORMATION/CLM)

=> s l23 and (HIV/clm or human immunodeficiency virus/clm)
          4723 HIV/CLM
         75340 HUMAN/CLM
          1979 IMMUNODEFICIENCY/CLM
         12789 VIRUS/CLM
          1461 HUMAN IMMUNODEFICIENCY VIRUS/CLM
                 ((HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)/CLM)
L24        248 L23 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)

=> s l24 and ay<2001
       3178100 AY<2001
L25        115 L24 AND AY<2001

=> d l25,ti,1-115

L25  ANSWER 1 OF 115  USPATFULL on STN
TI     Vectors that repress heterologous promoter activity

L25  ANSWER 2 OF 115  USPATFULL on STN
TI     Encapsulation of components into edible products

L25  ANSWER 3 OF 115  USPATFULL on STN
TI     Production of recombinant respiratory syncytial viruses expressing
       immune modulatory molecules

L25  ANSWER 4 OF 115  USPATFULL on STN
TI     Use of triplex structure DNA in transferring nucleotide sequences

L25  ANSWER 5 OF 115  USPATFULL on STN
TI     Device and method for detecting polyvalent substances

L25  ANSWER 6 OF 115  USPATFULL on STN
TI     Helper-free rescue of recombinant negative strand RNA virus

L25  ANSWER 7 OF 115  USPATFULL on STN
TI     Expression of HIV polypeptides and production of virus-like particles

L25  ANSWER 8 OF 115  USPATFULL on STN
TI     HIV capsid assembly-associated compositions and method

L25  ANSWER 9 OF 115  USPATFULL on STN
TI     Retroviral hybrid vectors pseudotyped with LCMV

L25  ANSWER 10 OF 115  USPATFULL on STN
TI     Immune responses to hiv

L25  ANSWER 11 OF 115  USPATFULL on STN
TI     Constitutive expression of non-infectious HIV-like particles

L25  ANSWER 12 OF 115  USPATFULL on STN
TI     Fusion protein delivery system and uses thereof

L25  ANSWER 13 OF 115  USPATFULL on STN
TI     Anigenically-marked non-infectious retrovirus-like particles

L25  ANSWER 14 OF 115  USPATFULL on STN
TI     Non-infectious, immunogenic, human immunodeficiency virus-like
```

particles derived of long terminal repeats and a functional pol coding region

L25 ANSWER 15 OF 115 USPATFULL on STN
TI Anti-viral vectors

L25 ANSWER 16 OF 115 USPATFULL on STN
TI Methods for detecting **human immunodeficiency virus** nucleic acid

L25 ANSWER 17 OF 115 USPATFULL on STN
TI Lentiviral vectors

L25 ANSWER 18 OF 115 USPATFULL on STN
TI Antigentically-marked non-infectious retrovirus-like particles

L25 ANSWER 19 OF 115 USPATFULL on STN
TI PRENATAL SCREENING

L25 ANSWER 20 OF 115 USPATFULL on STN
TI Fusion protein delivery system and uses thereof

L25 ANSWER 21 OF 115 USPATFULL on STN
TI Use of recombinant parainfluenza viruses (PIVs) as vectors to protect against infection and disease caused by PIV and other human pathogens

L25 ANSWER 22 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido bicyclic heteroaryl hydroxamic acids as matrix metalloproteinase and tace inhibitors

L25 ANSWER 23 OF 115 USPATFULL on STN
TI Retrovirus like particles made non infectious by a plurality of mutations

L25 ANSWER 24 OF 115 USPATFULL on STN
TI Polypeptides fused with alfalfa mosaic virus or ilarvirus capsid

L25 ANSWER 25 OF 115 USPATFULL on STN
TI LENTIVIRUS BASED VECTOR AND VECTOR SYSTEM

L25 ANSWER 26 OF 115 USPATFULL on STN
TI N-hydroxy-2-(alkyl, aryl, or heteroaryl sulfanyl, sulfinyl or sulfonyl)-3-substituted alkyl, aryl or heteroarylamides as matrix metalloproteinase inhibitors

L25 ANSWER 27 OF 115 USPATFULL on STN
TI Retroviral hybrid vectors pseudotyped with LCMV

L25 ANSWER 28 OF 115 USPATFULL on STN
TI THERAPEUTICALLY ACTIVE COMPOUNDS BASED ON INDAZOLE BIOISOSTERE REPLACEMENT OF CATECHOL IN PDE4 INHIBITORS

L25 ANSWER 29 OF 115 USPATFULL on STN
TI METHODS AND COMPOSITIONS FOR POLYPEPTIDE ENGINEERING

L25 ANSWER 30 OF 115 USPATFULL on STN
TI FELINE IMMUNODEFICIENCY VIRUS GENE THERAPY VECTORS

L25 ANSWER 31 OF 115 USPATFULL on STN
TI Induction of cytotoxic T-lymphocyte responses

L25 ANSWER 32 OF 115 USPATFULL on STN
TI Fusion protein delivery system and uses thereof

L25 ANSWER 33 OF 115 USPATFULL on STN
TI Stabilized oligonucleotides and their use

L25 ANSWER 34 OF 115 USPATFULL on STN
TI N-hydroxy-2-(Alkyl,Aryl or Heteroaryl sulfanyl, sulfinyl or sulfonyl) 3-substituted alkyl, aryl or heteroarylamides as matrix metalloproteinase inhibitors

L25 ANSWER 35 OF 115 USPATFULL on STN
TI Diagnostic kits comprising genetically engineered **human immunodeficiency virus**-like particles containing heterologous antigenic markers

L25 ANSWER 36 OF 115 USPATFULL on STN
TI N-Hydroxy-2-(Alkyl, Aryl, or Heteroaryl, Sulfanyl, Sulfinyl or Sulfonyl)-3-Substituted Alkyl, Aryl or Heteroarylamides as Matrix Metalloproteinase inhibitors

L25 ANSWER 37 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido heteroaryl hydroxamic acids as matrix metalloproteinase and TACE inhibitors

L25 ANSWER 38 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido bicyclic heteroaryl hydroxamic acids as matrix metalloproteinase and TACE inhibitors

L25 ANSWER 39 OF 115 USPATFULL on STN
TI Retroviral vectors

L25 ANSWER 40 OF 115 USPATFULL on STN
TI Diagnostic assay method and kit for the detection of HHV-8 infection

L25 ANSWER 41 OF 115 USPATFULL on STN
TI Genetically engineered retroviral vector particles capable of infecting non-dividing cells

L25 ANSWER 42 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido bicyclic heteroaryl hydroxamic acids as matrix metalloproteinase and tace inhibitors

L25 ANSWER 43 OF 115 USPATFULL on STN
TI Nucleic acids containing modified **human immunodeficiency virus** genomes devoid of long terminal repeats encoding non-infectious, replication-deficient, immunogenic retrovirus-like particles

L25 ANSWER 44 OF 115 USPATFULL on STN
TI Antigenically-marked non-infectious retrovirus-like particles

L25 ANSWER 45 OF 115 USPATFULL on STN
TI N-hydroxy-2-(alkyl, aryl, or heteroaryl, sulfanyl, sulfinyl or sulfonyl)-3-substituted alkyl, aryl or heteroarylamides as matrix metalloproteinase inhibitors

L25 ANSWER 46 OF 115 USPATFULL on STN
TI Lentiviral vectors derived from SIVagm, methods for their preparation and their use for gene transfer into mammalian cells

L25 ANSWER 47 OF 115 USPATFULL on STN
TI In vitro diagnostic methods and kits for the detection of **HIV**-2-specific antibodies

L25 ANSWER 48 OF 115 USPATFULL on STN
TI Virus protein antigens of the JC virus

L25 ANSWER 49 OF 115 USPATFULL on STN
TI Lentiviral vectors

L25 ANSWER 50 OF 115 USPATFULL on STN
TI Cell transformation vector comprising an **HIV**-2 packaging site nucleic

acid and an HIV-1 GAG protein

L25 ANSWER 51 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido heteroaryl hydroxamic acids as matrix metalloproteinase and tace inhibitors

L25 ANSWER 52 OF 115 USPATFULL on STN
TI N-hdroxy-2-(alkyl, aryl, or heteroaryl, sulfanyl, sulfinyl or sulfonyl)-3-substituted alkyl, aryl or heteroarylamides as matrix metalloproteinase inhibitors

L25 ANSWER 53 OF 115 USPATFULL on STN
TI DNA expression systems based on alphaviruses

L25 ANSWER 54 OF 115 USPATFULL on STN
TI N-Hydroxy-2-(alkyl, aryl, or heteroaryl sulfanyl, sulfinyl or sulfonyl)-3-substituted alkyl, aryl or heteroarylamides as matrix metalloproteinase inhibitors

L25 ANSWER 55 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamide heteroarly hydroxamic acids as matrix metalloproteinase and TACE inhibitors

L25 ANSWER 56 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido heteroaryl hydroxamic acids as matrix metaloproteinase and tace inhibitors

L25 ANSWER 57 OF 115 USPATFULL on STN
TI Constitutive expression of non-infectious **HIV**-like particles

L25 ANSWER 58 OF 115 USPATFULL on STN
TI Chimeric Gag pseudovirions

L25 ANSWER 59 OF 115 USPATFULL on STN
TI **Human immunodeficiency virus** type 1 nucleic acids devoid of long terminal repeats capable of encoding for non-infectious, immunogenic, retrovirus-like particles

L25 ANSWER 60 OF 115 USPATFULL on STN
TI Recombinant **HIV** and modified packaging cells and method for using

L25 ANSWER 61 OF 115 USPATFULL on STN
TI Self-assembled, defective, nonself-propagating viral particles

L25 ANSWER 62 OF 115 USPATFULL on STN
TI Expression vectors encoding recombinant proteins comprising a VPR/VPX virion incorporation domain for targeting into **HIV**-1 or **HIV**-2 virions

L25 ANSWER 63 OF 115 USPATFULL on STN
TI Polypeptides fused with alfalfa mosaic virus or ilarvirus capsid proteins

L25 ANSWER 64 OF 115 USPATFULL on STN
TI Immunofluorescence assay for the detection of antibodies using recombinant antigens in insoluble form

L25 ANSWER 65 OF 115 USPATFULL on STN
TI Methods for the detection of **HIV**-specific immune responses employing non-infectious, non-replicating, IIV retrovirus-like particles containing heterologous antigenic markers

L25 ANSWER 66 OF 115 USPATFULL on STN
TI Non-infectious, replication-impaired, immunogenic **human immunodeficiency virus** type 1 retrovirus-like particles with multiple genetic deficiencies

L25 ANSWER 67 OF 115 USPATFULL on STN
TI Preparation and use of β-sulfonamido hydroxamic acids as matrix metalloproteinase and TACE inhibitors

L25 ANSWER 68 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido heteroaryl hydroxamic acids as matrix metalloproteinase and tace inhibitors

L25 ANSWER 69 OF 115 USPATFULL on STN
TI Non-infectious, replication-defective, self-assembling **HIV**-1 viral particles containing antigenic markers in the gag coding region

L25 ANSWER 70 OF 115 USPATFULL on STN
TI Preparation and use of ortho-sulfonamido aryl hydroxamic acids as matrix metalloproteinase and tace inhibitors

L25 ANSWER 71 OF 115 USPATFULL on STN
TI Non-infectious **HIV** particles and uses therefor

L25 ANSWER 72 OF 115 USPATFULL on STN
TI Nucleic acid molecules encoding non-infectious, non-replicating, **HIV** retrovirus-like particles containing heterologous antigenic anchor sequences

L25 ANSWER 73 OF 115 USPATFULL on STN
TI Isolation of novel **HIV**-2 proviruses

L25 ANSWER 74 OF 115 USPATFULL on STN
TI Genetically engineered **human immunodeficiency virus**-like particles with modified chimeric envelope glycoproteins containing influenza virus transmembrane spanning domains

L25 ANSWER 75 OF 115 USPATFULL on STN
TI Induction of neutralizing antibody against viral infection by synergy between virus envelope glycoprotein and peptides corresponding to neutralization epitopes of the glycoprotein

L25 ANSWER 76 OF 115 USPATFULL on STN
TI Modulation of gene expression through interference with RNA secondary structure

L25 ANSWER 77 OF 115 USPATFULL on STN
TI Nucleic acids encoding for non-infectious, replication-defective, self-assembling **HIV**-1 viral particles containing antigenic markers in the gag coding region

L25 ANSWER 78 OF 115 USPATFULL on STN
TI Methods, kits, and probes for diagnosing **HIV**-2

L25 ANSWER 79 OF 115 USPATFULL on STN
TI Non-infectious **HIV** particles and uses therefor

L25 ANSWER 80 OF 115 USPATFULL on STN
TI Chimeric proteins comprising a Vpr/Vpx virion incorporation domain for targeting into **HIV**-1 or **HIV**-2 virions

L25 ANSWER 81 OF 115 USPATFULL on STN
TI Alphavirus vector constructs

L25 ANSWER 82 OF 115 USPATFULL on STN
TI Compositions and methods for determining anti-viral drug susceptibility and resistance and anti-viral drug screening

L25 ANSWER 83 OF 115 USPATFULL on STN
TI In vitro diagnostic assays for the detection of **HIV**-1 or **HIV**-2 employing viral-specific antigens and antibodies

L25 ANSWER 84 OF 115 USPATFULL on STN
TI High efficiency translation of mRNA molecules

L25 ANSWER 85 OF 115 USPATFULL on STN
TI High efficiency translation of mRNA molecules

L25 ANSWER 86 OF 115 USPATFULL on STN
TI Self assembled, defective, nonself-propagating viral particles

L25 ANSWER 87 OF 115 USPATFULL on STN
TI Screening method for the identification of compounds capable of abrogation **HIV**-1 gag-cyclophilin complex formation

L25 ANSWER 88 OF 115 USPATFULL on STN
TI Chimeric retrovial expression vectors and particles containing a simple retroviral long terminal repeat, BLV or **HIV** coding regions and cis-acting regulatory sequences, and an RNA translational enhancer with internal ribsome entry site

L25 ANSWER 89 OF 115 USPATFULL on STN
TI Recombinant **HIV** and modified packaging cells and method for treating acquired immune deficiency syndrome

L25 ANSWER 90 OF 115 USPATFULL on STN
TI Reversible flow chromatographic binding assay

L25 ANSWER 91 OF 115 USPATFULL on STN
TI Self-assembled, defective, non-self-propagating lentivirus particles

L25 ANSWER 92 OF 115 USPATFULL on STN
TI Self assembled defective non-self propagating lentiviral particles

L25 ANSWER 93 OF 115 USPATFULL on STN
TI Chimeric envelope proteins for viral targeting

L25 ANSWER 94 OF 115 USPATFULL on STN
TI Induction of cytotoxic T-lymphocyte responses

L25 ANSWER 95 OF 115 USPATFULL on STN
TI Nucleic acids encoding mutated **human immunodeficiency virus** matrix proteins

L25 ANSWER 96 OF 115 USPATFULL on STN
TI **HIV** nucleocapsid protein capture assay and method of use

L25 ANSWER 97 OF 115 USPATFULL on STN
TI Methods and compositions for inhibiting production of replication competent virus

L25 ANSWER 98 OF 115 USPATFULL on STN
TI Sandwich hybridization assays using very short capture probes noncovalently bound to a hydrophobic support

L25 ANSWER 99 OF 115 USPATFULL on STN
TI Method for isolation of unclipped **HIV** envelope protein

L25 ANSWER 100 OF 115 USPATFULL on STN
TI Diagnastic method and test kit for the serological detection of the AIDS virus

L25 ANSWER 101 OF 115 USPATFULL on STN
TI Surgical glove that protects against infection by providing heat in response to penetration thereof by a medical instrument and method therefor

L25 ANSWER 102 OF 115 USPATFULL on STN
TI Fusion glycoproteins

L25 ANSWER 103 OF 115 USPATFULL on STN
TI Self assembled, defective, non-self-propagating lentivirus particles

L25 ANSWER 104 OF 115 USPATFULL on STN
TI Method for the identification of compounds capable of abrogating **human immunodeficiency virus** (**HIV**) infection of dendritic cells and T-lymphocytes

L25 ANSWER 105 OF 115 USPATFULL on STN
TI Multiple branch peptide constructions for use against **HIV**

L25 ANSWER 106 OF 115 USPATFULL on STN
TI Self-assembled, defective, non-self-propagating lentivirus particles

L25 ANSWER 107 OF 115 USPATFULL on STN
TI Autonomous parvovirus gene delivery vehicles and expression vectors

L25 ANSWER 108 OF 115 USPATFULL on STN
TI Chimeric immunogenic gag-V3 virus-like particles of the **human immunodeficiency virus** (**HIV**)

L25 ANSWER 109 OF 115 USPATFULL on STN
TI Peptides of **human immunodeficiency virus** type 2 (**HIV**-2) and in vitro diagnostic methods and kits employing the peptides for the detection of **HIV**-2

L25 ANSWER 110 OF 115 USPATFULL on STN
TI Fusion proteins and particles

L25 ANSWER 111 OF 115 USPATFULL on STN
TI Chimeric **HIV**-2 gag particles

L25 ANSWER 112 OF 115 USPATFULL on STN
TI Self-assembling replication defective hybrid virus particles

L25 ANSWER 113 OF 115 USPATFULL on STN
TI Methods and kits for diagnosing **human immunodeficiency virus** type 2(**HIV**-2)

L25 ANSWER 114 OF 115 USPATFULL on STN
TI Diagnostic method and composition for early detection of **HIV** infection

L25 ANSWER 115 OF 115 USPATFULL on STN
TI T-cell lymphotrophic virus protein and assay

=> d l25,cbib,ab,clm,8,58,57,71,87,92,108

L25 ANSWER 8 OF 115 USPATFULL on STN
2003:190664 **HIV** capsid assembly-associated compositions and method.
Lingappa, Jaisri R., San Francisco, CA, United States
Lingappa, Vishwanath R., San Francisco, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 6593103 B1 20030715
APPLICATION: US 1998-20144 19980206 (9) <--
PRIORITY: US 1997-39309P 19970207 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A cell-free method for translation and assembly of retroviral, particularly **HIV**, capsid and capsid intermediates is disclosed. Also disclosed are novel **HIV** capsid assembly intermediates and novel host proteins which bind to such assembly intermediates. The invention also

includes a screening method for compounds that direct retrovirus capsid assembly, and a method of treating **HIV** using compounds which inhibit the **HIV** capsid assembly pathway.

CLM What is claimed is:

1. A cell-free system for translation and **assembly** of an **HIV capsid**, comprising: a cell-free translation mixture which contains a cell-free extract comprising a eukaryotic cell component of a high speed pellet, amino acids, transfer RNA (tRNA), ribosomes, and an energy source, an mRNA molecule encoding a **Gag** Pr55 protein derived from **human immunodeficiency virus** (**HIV**), and myristoyl coenzyme A, wherein said eukaryotic cell component is characterized by insensitivity to a concentration of at least 0.5% (wt/vol) octaethyleneglycol mono n-dodecyl ether detergent, wherein said eukaryotic cell component comprises a host **assembly** protein which is a 68 kilodalton that is present in a detergent treated high speed pellet of a wheat germ extract or a protein having the structure of said 68 kilodalton protein, and wherein said protein provides for **capsid assembly** in said cell-free system when said **HIV** Pr55 mRNA **Gag** protein is wild type.

2. The cell-free translation system according to claim 1, which further includes a detergent-sensitive fraction derived from eukaryotic cell membranes.

3. The cell-free translation system according to claim 1, wherein said eukaryotic cell component comprises one or more host **assembly** protein that is immunoteactive with antibody 23c and forms one or more complex with said **Gag** protein.

4. The cell-free translation system according to claim 1, which further includes (i) a DNA molecule which encodes **HIV Gag** Pr55, (ii) an RNA polymerase for synthesizing said mRNA, and (iii) sufficient concentrations of nucleotides ATP, UTP, GTP, and CTP to support such mRNA synthesis.

5. The cell-free translation system according to claim 1, wherein said **HIV Gag** mRNA encodes a mutant defective in **assembly**.

6. The cell-free translation system according to claim 1, wherein said concentration of myristoyl coenzyme A is between about 5 and 30 micromolar.

7. The cell free system according to claim 1, wherein said concentration of myristoyl coenzyme A is between about 0.01 and 100 micromolar.

8. A method for producing an **HIV capsid** intermediate in a cell-free system, said method comprising: adding to a cell-free protein translation mixture which contains a cell-free extract comprising a eukaryotic cell component of a high speed pellet, amino acids, transfer RNA (tRNA), ribosomes, and an energy source: (i) an mRNA molecule encoding an **HIV** Pr55 mRNA **Gag** protein, and (ii) a concentration of myristoyl coenzyme A that is greater than about 0.1 micromolar, to form a reaction mixture; wherein said eukaryotic cell component is characterized by insensitivity to a concentration of at least 0.5% (wt/vol) octaethyleneglycol mono n-dodecyl ether detergent, wherein said eukaryotic cell component comprises a host **assembly** protein which is a 68 kilodalton that is present in a detergent treated high speed pellet of a wheat germ extract or a protein having the structure of said 68 kilodalton host **assembly** protein, and wherein said protein provides for **capsid assembly** in said cell-free system when said **HIV** Pr55 mRNA **Gag** protein is wild type; and incubating said reaction mixture for a period of time sufficient to assemble **Gag** Pr55 mRNA translation products into one or more **HIV capsid** intermediate.

9. The method according to claim 8, wherein said cell-free extract comprises a detergent-sensitive fraction derived from eukaryotic cell membranes.

10. The method according to claim 8, wherein said host **assembly** protein comprises a peptide region having the sequence shown in SEQ.ID.NO.: 2 and C-terminal epitope LDD-COOH.

11. The method according to claim 8, which further includes adding to said reaction a **Gag** Pr55 DNA transcript and a transcription mixture containing an RNA polymerase and ribonucleotides ATP, UTP, GTP and CTP effective to produce said **Gag** mRNA in said cell-free mixture.

L25 ANSWER 58 OF 115 USPATFULL on STN
2000:101880 Chimeric Gag pseudovirions.
Tobin, Gregory J., Frederick, MD, United States
Gonda, Matthew A., Newtown Square, PA, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6099847 20000808
APPLICATION: US 1997-857385 19970515 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides, inter alia, recombinant chimeric nucleic acids encoding a Gag-fs-fusion partner fusion protein; a pseudovirion comprising a retroviral Gag protein and a fusion partner, wherein the fusion partner is present in a Gag-fs-fusion partner fusion protein; an immunogenic composition comprising a pseudovirion; a Gag-fs-fusion partner fusion protein; and a method of making the pseudovirions of the present invention.
CLM What is claimed is:
1. A recombinant chimeric nucleic acid, comprising: a retroviral **gag** sequence; a target nucleic acid sequence derived from a nucleic acid encoding a fusion partner selected from the group consisting of Env, an interleukin, TNF, GM/CSF, a nonretroviral viral antigen and a cancer antigen; wherein the **gag** and target sequences are transcribed from a single start site of transcription, and wherein the **gag** and target sequences are in different reading frames; and, a frame-shift site.

2. The recombinant chimeric nucleic acid of claim 1, wherein the target nucleic acid sequence is derived from a nucleic acid encoding a fusion partner selected from the group consisting of IL-1, IL-2, IL-4, IL-6, MART-1, gp 100, tyrosinase, bcl-1, bcl-2, c-myc, int-2, hst-1, ras, p53, prostate-specific membrane antigen, papilloma virus protein L1, protein kinase C., and G proteins.

3. The recombinant chimeric nucleic acid of claim 1, wherein the frame shift site is derived from a site selected from the group consisting of a retroviral frame shift site, a retrotransposon frame shift site, a human astrovirus frame shift site, a mouse intracisternal **particle** frame shift site, an HERV frame shift site, a Ty element frame shift site, and an optimized synthetic frameshift site.

4. A recombinant chimeric **gag**-env nucleic acid, comprising: a retroviral **gag** sequence; a retroviral env sequence; wherein the **gag** and env sequences are transcribed from a single start site of transcription, and wherein the **gag** and env sequences are in different reading frames; and, a retroviral frame-shift site derived from a retroviral **gag**-pol frame shift site.

5. The recombinant nucleic acid of claim 4, wherein the env sequence encodes approximately the carboxyl 65% of Env protein.

6. The recombinant nucleic acid of claim 4, wherein the nucleic acid further comprises a pol sequence.

7. The recombinant nucleic acid of claim 4, wherein the nucleic acid is a subsequence in a baculoviral vector.

8. The recombinant nucleic acid of claim 4, wherein the nucleic acid is competent to produce pseudovirions in an insect cell.

9. The recombinant nucleic acid of claim 4, wherein the nucleic acid is competent to produce pseudovirions in an insect cell, and wherein the nucleic acid hybridizes under stringent conditions to **HIV Gag**-fs-SU.

10. The recombinant nucleic acid of claim 4, wherein the nucleic acid is **HIV Gag**-fs-SU or a conservative variation thereof.

11. The recombinant nucleic acid of claim 4, wherein the nucleic acid is **HIV Gag**-fs-SU.

12. The recombinant nucleic acid of claim 4, wherein the nucleic acid is a subsequence in a baculoviral vector, wherein the vector is competent to transduce an insect cell.

13. The recombinant nucleic acid of claim 4, wherein the **gag** and env sequences are derived from **HIV**.

14. The recombinant nucleic acid of claim 4, wherein the nucleic acid further comprises a polyhedrin promoter.

15. The recombinant nucleic acid of claim 4, wherein the nucleic acid further comprises an SV 40 polyadenylation site.

16. A pseudovirion comprising a retroviral **Gag** protein and a fusion partner, wherein the fusion partner is present in a **Gag**-fs-fusion partner fusion protein.

17. The pseudovirion of claim 16, wherein the fusion partner is derived from a protein selected from the group consisting of an interleukin, TNF, GM/CSF, a nonretroviral viral antigen, a cancer antigen and a molecule involved in signal transduction.

18. The pseudovirion of claim 17, wherein the fusion partner is derived from a protein selected from the group consisting of IL-1, IL-2, IL-4, IL-6, MART-1, gp 100, tyrosinase, bcl-1, bcl-2, c-myc, int-2, hst-1, ras, p53, prostate-specific membrane antigen, papilloma virus protein L1, protein kinase C., and G proteins.

19. The pseudovirion of claim 16, wherein the fusion partner is derived from a retroviral Env protein.

20. The pseudovirion of claim 16, wherein the pseudovirion is noninfectious.

21. The pseudovirion of claim 19, wherein the Env protein domain is present primarily in the interior of the pseudovirion.

22. The pseudovirion of claim 19, wherein the **Gag**-fs-Env fusion protein is the **Gag**-fs-SU fusion protein, or a conservative modification thereof.

23. The pseudovirion of claim 19, wherein the **Gag**-fs-Env fusion protein is the **Gag**-fs-SU fusion protein.

24. The pseudovirion of claim 19, wherein the Env fusion partner is present in a **Gag**-fs-Env fusion protein, and wherein **Gag** protein is separately present in the fusion protein and as an independent protein.

25. The pseudovirion of claim 19, wherein the pseudovirion is made by transducing an insect cell with a baculovirus vector, which vector encodes a **Gag**-fs-Env protein.

26. The pseudovirion of claim 25, wherein the pseudovirion, when administered as an immunogenic composition in mice, elicits a CTL response against Env, but does not elicit antibodies which recognize Env.

27. An immunogenic composition comprising a pseudovirion comprising a retroviral **Gag** protein and a retroviral fusion partner, wherein the fusion partner is present in a **Gag**-fs-fusion partner fusion protein and wherein the fusion partner is derived from a retroviral Env protein.

28. The immunogenic composition of claim 27, wherein the immunogenic composition, when administered to mice, elicits a CTL response against Env, but does not elicit antibodies against Env.

29. A particulate vaccine comprising a pseudovirion comprising a retroviral **Gag** protein and a retroviral fusion partner, wherein the fusion partner is present in a **Gag**-fs-fusion partner fusion protein and wherein the fusion partner is derived from a retroviral Env protein.

30. The particulate vaccine of claim 29, wherein the vaccine, when administered to mice, elicits a CTL response against Env, but does not elicit antibodies against Env.

31. A fusion protein comprising a retroviral **Gag** sequence, a translation reading frame switching sequence and a fusion partner.

32. The fusion protein of claim 31, wherein the fusion partner is a retroviral Env amino acid subsequence.

33. The fusion protein of claim 31, wherein the fusogenic partner is selected from the group consisting of Env, an interleukin, TNF, GM/CSF, a nonretroviral viral antigen, a cancer antigen and a molecule involved in signal transduction.

34. The fusion protein of claim 31, wherein the fusogenic partner is selected from the group consisting of IL-1, IL-2, IL-4, IL-6, MART-1, gp 100, tyrosinase, bcl-1, bcl-2, c-myc, int-2, hst-1, ras, p53, prostate-specific membrane antigen, papilloma virus protein L1, protein kinase C. and G proteins.

35. The fusion protein of claim 32, wherein the Env amino acid subsequence comprises the carboxyl 65% of a retroviral Env protein.

36. The fusion protein of claim 32, wherein the Env amino acid subsequence is derived from **HIV**.

37. The fusion protein of claim 31, wherein the translation reading frame switching sequence comprises sequences derived from the N-terminus of a retroviral Pol protein.

38. A method of making a pseudovirion comprising expressing a nucleic acid encoding a **Gag**-fs-fusion partner fusion protein in a cell, wherein the cell translates the nucleic acid into a first protein comprising a **Gag** sequence, and a second protein comprising a **gag** sequence and a fusogenic partner.

39. The method of claim 38, wherein the fusogenic partner comprises an env sequence.

40. The method of claim 38, wherein the cell is an insect cell.

41. The method of claim 38, wherein the method further comprises the step of purifying the pseudovirion.

42. A pseudovirion comprising a retroviral **Gag** protein and a fusion partner, wherein the fusion partner is present in **Gag**-fs-fusion

protein fusion protein and wherein the fusion partner is derived from retroviral Env protein.

43. A fusion protein comprising a retroviral **Gag** sequence, a translation reading frame switching sequence and a retroviral Env amino acid subsequence.

L25 ANSWER 57 OF 115 USPATFULL on STN
2000:124799 Constitutive expression of non-infectious **HIV**-like particles.
Rovinski, Benjamin, Thornhill, Canada
Yao, Fei-Long, North York, Canada
Cao, Shi Xian, Etobicoke, Canada
Connaught Laboratories Limited, North York, Canada (non-U.S. corporation)
US 6121021 20000919
APPLICATION: US 1997-991773 19971216 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Non-infectious, non-replicating immunogenic **HIV**-like particles are produced by stable longn-term constitutive expression in mammalian cells by eliminating elements toxic to the mammalian cells. An expression vector contains a nucleic acid molecule comprising a modified **HIV** genome devoid of long terminal repeats and wherein Tat and vpr sequences are functionally disabled and a constitutive promoter operatively connected to the modified **HIV** genome for constitutive expression of the modified genome to produce the **HIV**-like particles.
CLM What is claimed is:
1. A nucleic acid molecule, comprising a modified **HIV** genome devoid of long terminal repeats and wherein vpr and tat sequences are functionally disabled and a constitutive promoter operatively connected to said modified **HIV** genome for constitutive expression of said modified genome to produce non-infectious, non-replicating and immunogenic **HIV**-like **particles**.

2. The nucleic acid molecule of claim 1 wherein said vpr and tat sequences are functionally disabled by the insertion of stop codons therein preventing expression of the respective encoded gene products.

3. The nucleic acid molecule of claim 1 wherein the **HIV** genome is further modified by replacing the signal peptide encoding sequence of gp120 by the signal peptide encoding sequence of glycoprotein D of herpes simplex virus.

4. The nucleic acid molecule of claim 1 wherein the env gene encodes an env gene product from a primary **HIV**-1 isolate.

5. The nucleic acid molecule of claim 1 wherein said **HIV** genome is further modified to effect reduction in **gag**-dependent RNA packaging of the **gag** gene product.

6. The nucleic acid molecule of claim 5 wherein said reduction in **gag**-dependent RNA packaging of the **gag** gene product is effected by replacing Cys 392 and Cys 395 of the **gag** gene product of **HIV**-1 LAI isolate, or the corresponding amino acids of another **HIV** isolate, by serine.

7. The nucleic acid molecule of claim 1 wherein said **HIV** genome is further modified to substantially eliminate reverse transcriptase activity, integrase activity and RNAse activity.

8. The nucleic acid molecule of claim 7 wherein a BalI--BalI portion of pol gene is deleted between nucleotides 2655 and 4507 of the LAI isolate of **HIV**-1 or the corresponding portion of the pol gene of another **HIV**-1 isolate.

9. The nucleic acid molecule of claim 1 wherein the constitutive

promoter is the human immediate early cytomegalovirus promoter.

10. The nucleic acid molecule of claim 9 wherein an expression enhancing sequence is provided between said promoter and said modified genome.

11. The nucleic acid molecule of claim 10 wherein said expression enhancing sequence is the human cytomegalovirus Intron A sequence.

12. An expression vector comprising the nucleic acid molecule of claim 1.

13. The expression vector of claim 12 which is plasmid pCMVgDtat^{--} vpr^{--} as shown in FIG. 2A as deposited under ATCC Deposit No. 209446.

14. A method of obtaining a non-infectious, non-replicating, immunogenic **HIV**-like **particle**, which comprises: incorporating into an expression vector a nucleic acid molecule comprising a modified **HIV** genome devoid of long terminal repeats and wherein vpr and tat sequences are functionally disabled and a constitutive promoter operatively connected to said modified **HIV** genome, introducing the expression vector into mammalian cells, and constitutively expressing the nucleic acid molecule in said cells to stably produce non-infectious, non-replicating, immunogenic **HIV**-like **particles**.

15. The method of claim 14 wherein said vpr and tat sequences are functionally disabled by the insertion of stop codons therein preventing expression of the respective encoded gene products.

16. The method of claim 14 wherein the **HIV** genome is further modified by replacing the signal peptide encoding sequence of gp120 by the signal peptide encoding sequence of glycoprotein D of herpes simplex virus.

17. The method of claim 14 wherein the env gene encodes an env gene product from a primary **HIV**-1 isolate.

18. The method of claim 14 wherein said **HIV** genome is further modified to effect reduction in **gag**-dependent RNA packaging of the **gag** gene product.

19. The method of claim 18 wherein said reduction in **gag**-dependent RNA packaging of the **gag** gene product is effected by replacing Cys 392 and Cys 395 of the **gag** gene product of **HIV**-1 LAI isolate, or the corresponding amino acids of another **HIV** isolate, by serine.

20. The method of claim 14 wherein said **HIV** genome is further modified to substantially eliminate reverse transcriptase activity, integrase activity and RNAse activity.

21. The method of claim 20 wherein a BalI--BalI portion of pol gene is deleted between nucleotides 2655 and 4507 of the LAI isolate of **HIV**-1 or the corresponding portion of the pol gene of another **HIV**-1 isolate.

22. The method of claim 14 wherein the constitutive promoter is the human immediate early cytomegalovirus promoter.

23. The method of claim 22 wherein an expression enhancing sequence is provided between said promoter and said modified genome.

24. The method of claim 23 wherein said expression enhancing sequence is the human cytomegalovirus Intron A sequence.

25. The method of claim 24 wherein expression of the nucleic acid molecule also is induced.

26. The method of claim 14 wherein said expression vector is plasmid

pCMVΔ8.2 ΔVpr as deposited with ATCC under Deposit No. 209446 and as shown in FIG. 2A.

27. A non-infectious, non-replicating immunogenic **HIV**-like **particle** lacking Tat and Vpr and producible by the method of claim 14.

28. An immunogenic composition, comprising the non-infectious, non-replicating immunogenic **HIV**-like **particle** claimed in claim 27 and a physiologically-acceptable carrier therefor.

L25 ANSWER 71 OF 115 USPATFULL on STN
1999:75316 Non-infectious **HIV** particles and uses therefor.
Aldovini, Anna, Winchester, MA, United States
Young, Richard A., Winchester, MA, United States
Feinberg, Mark B., San Francisco, CA, United States
Trono, Didier, Solana Beach, CA, United States
Baltimore, David, New York, NY, United States
Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
US 5919458 19990706
APPLICATION: US 1995-477081 19950607 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention related to constructs comprising mutant **HIV** genomes having an alteration in a nucleotide sequence which is critical for genomic RNA packaging and non-infectious, immunogenic **HIV** particles produced by expression of these constructs in mammalian cells. Cell lines which stably produce non-infectious, immunogenic **HIV** particles are also included. Prophylactic and therapeutic vaccines, diagnostic reagents, and related methods are further described.
CLM What is claimed is:
1. An immunogen comprising non-infectious **HIV particles** or antigenic portions thereof, in a physiologically acceptable vehicle, wherein the non-infectious **HIV particles** or antigenic portions thereof have a protein composition similar to that of a wild type **HIV** and lack **HIV** genomic RNA and are produced by a method comprising the steps of: a) transfecting a mammalian cell line with a construct which encodes non-infectious **HIV particles** and, when expressed in mammalian cells, produces only non-infectious **HIV particles** which have a protein composition similar to that of wild type **HIV** and lack **HIV** genomic RNA, the construct having an alteration of the wild type **HIV** genome, the alteration selected from the group consisting of: 1) deletion in the ψ site, wherein the deletion is selected from the group consisting of: i) deletion of nucleotides 293 to 331, inclusive, and ii) deletion of nucleotides 293 to 313, inclusive; 2) an alteration in the **gag** gene which results in an alteration of the amino acid sequence of the encoded protein, the amino acid alteration selected from the group consisting of: i) substitution of tyrosine for the first two cysteines of the 5' CysHis box; ii) substitution of tyrosine for the first two cysteines of the 3' CysHis box; iii) substitution of tyrosine for the first two cysteines of both CysHis boxes; iv) deletion of both CysHis boxes and the amino acid sequence between them; and v) alteration of the length of the amino acid sequence between the two CysHis boxes; and 3) deletion in the ψ site and an alteration in the **gag** gene which results in an alteration of the amino acid sequence of the encoded protein, wherein: i) the deletion in the ψ site is selected from the group consisting of: (a) deletion of nucleotides 293 to 331, inclusive; and (b) deletion of nucleotides 293 to 313, inclusive; and ii) the alteration in the **gag** gene is selected from the group consisting of: (a) substitution of tyrosine for the first two cysteines of the 5' CysHis box, (b) substitution of tyrosine for the first two cysteines of the 3' CysHis box; (c) substitution of tyrosine for the first two cysteines of both CysHis boxes; (d) deletion of both CysHis boxes and the amino acid sequence between them; and (e) alteration of the length of the amino acid sequence between the two CysHis boxes; and b) expressing the

cultured in the cell line, thereby producing non-infectious **HIV particles** which have a protein composition similar to that of wild type **HIV** and lack **HIV** genomic RNA.

2. An immunogen comprising non-infectious **HIV**-1 virions or antigenic portions thereof, in a physiologically acceptable vehicle, wherein the non-infectious **HIV** virions or antigenic portions thereof have a protein composition similar to that of wild type **HIV** and lack **HIV** genomic RNA and are produced by culturing a mammalian cell line which stably produces non-infectious **HIV particles** which have a protein composition similar to that of wildtype **HIV** and lack **HIV** genomic RNA, wherein the mammalian cell line comprises a mutant **HIV** genome stably integrated in the genome of said cell line and the mutant **HIV** genome comprises an alteration of the wild type **HIV** genome, the alteration selected from the group consisting of: 1) deletion in the ψ site, wherein the deletion is selected from the group consisting of: i) deletion of nucleotides 293 to 331, inclusive; and ii) deletion of nucleotides 293 to 313, inclusive; 2) an alteration in the **gag** gene which results in an alteration of the amino acid sequence of the encoded protein, the amino acid alteration selected from the group consisting of: i) substitution of tyrosine for the first two cysteines of the 5' CysHis box; ii) substitution of tyrosine for the first two cysteines of the 3' CysHis box; iii) substitution of tyrosine for the first two cysteines of both CysHis boxes; iv) deletion of both CysHis boxes and the amino acid sequence between them; and v) alteration of the length of the amino acid sequence between the two CysHis boxes; and 3) deletion in the ψ site and an alteration in the **gag** gene which results in an alteration of the amino acid sequence of the encoded protein, wherein: i) the deletion in the ψ site is selected from the group consisting of: (a) deletion of nucleotides 293 to 331, inclusive; and (b) deletion of nucleotides 293 to 313, inclusive; and ii) the alteration in the **gag** gene is selected from the group consisting of: (a) substitution of tyrosine for the first two cysteines of the 5' CysHis box; (b) substitution of tyrosine for the first two cysteines of the 3' CysHis box; (c) substitution of tyrosine for the first two cysteines of both CysHis boxes; (d) deletion of both CysHis boxes and the amino acid sequence between them; and (e) alteration of the length of the amino acid sequence between the two CysHis boxes, thereby producing non-infectious **HIV** virions which have a protein composition similar to that of wild type **HIV** and lack **HIV** genomic RNA.

3. The immunogen of claim 1, wherein the construct in step a) is selected from the group consisting of pA3HXB and pA4HXB.

4. The immunogen of claim 1, wherein the construct in step a) is selected from the group consisting of: a) pA15HXB; b) pA14HXB; c) pA14-15HXB; and d) pΔCH1-2HXB.

5. The immunogen of claim 1, wherein the construct in step a) is selected from the group consisting of pΔPAC1 and pΔPAC-Hygro.

6. The immunogen of claim 1, wherein the construct in step a) further comprises an alteration selected from the group consisting of: a) an alteration which results in substitution of the envelope precursor cleavage site with VVQGEEFAVG (SEQ ID NO:9); b) deletion of the primer binding site; and c) a combination of 1) an alteration which results in substitution of the envelope precursor cleavage site with VVQGEEFAVG and 2) deletion of the primer binding site.

7. The immunogen of claim 6, wherein the construct is selected from the group consisting of pΔPAC2 and pΔPAC3.

8. The immunogen of claim 1, wherein the construct in step a) further comprises an SV40 origin of replication.

9. The immunogen of claim 1, wherein the alteration of the wildtype **HIV** genome in the construct in step a) further comprises a selectable marker gene in place of the nef gene.

10. The immunogen of claim 9, wherein said selectable marker gene encodes a selectable marker which is selected from the group consisting of neomycin resistance, hygromycin resistance and dihydrofolate reductase.

11. The immunogen of claim 1, wherein the construct in step a) further comprises an **HIV** env gene from another **HIV** strain or isolate which is substituted for a native **HIV** env gene of the construct.

L25 ANSWER 87 OF 115 USPATFULL on STN
1998:75376 Screening method for the identification of compounds capable of abrogation **HIV**-1 gag-cyclophilin complex formation.
Luban, Jeremy, New York, NY, United States
Goff, Stephen P., Tenafly, NJ, United States
The Trustees of Columbia University in the City of New York, New York, NY, United States (U.S. corporation)
US 5773225 19980630
APPLICATION: US 1994-248357 19940524 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The **human immunodeficiency virus** type 1 (**HIV**-1) gag gene product is capable of directing the assembly of virion particles independent of other viral elements. The Gag protein also plays an important role during the early stages of viral replication. Employing the yeast two-hybrid system, a cDNA expression library was screened and two host proteins identified. These proteins, designated cyclophilins A and B (CyPsA and B), interacted specifically with the **HIV**-1 Gag polyprotein $Pr55^{gag}$. Glutathione S-transferase-CyP fusion proteins bind tightly to $Pr55^{gag}$ in vitro. Cyclosporin A (CsA) efficiently disrupts the Gag-CyPA binding interaction. The identification of novel compounds capable of abrogating this protein-protein interaction employing the disclosed screening assay will facilitate the development of **HIV**-1 antiviral agents.

CLM What is claimed is:
1. A method for identifying compounds capable of interfering with the **formation** of a complex between an **HIV**-1 **Gag** protein and a cyclophilin (CyP), which comprises the following steps: a) producing a CyP affinity fusion protein; b) pre-incubating a compound with the CyP affinity fusion protein of step (a); c) adding an **HIV**-1 **Gag** protein to the incubate of step (b) under conditions which permit **Gag** and the CyP affinity fusion protein to form a complex; d) contacting the incubate of step (c) with an affinity medium under conditions that enable the **Gag**-CyP affinity fusion protein complex to bind to said affinity medium; e) determining the amount of said **Gag**-cyclophilin affinity fusion protein complex **formation** by comparison to a control sample lacking said compound; wherein reduced binding of **HIV**-1 **Gag** to the cyclophilin affinity fusion protein is indicative of the ability of said compound to inhibit said complex **formation**.

2. The method of claim 1, wherein the CyP employed in the CyP affinity fusion protein is selected from the group consisting of CyP A, B, C, D, and combinations thereof.

3. The method of claim 1, wherein the CyP affinity fusion protein is a glutathione S-transferase-CyP (GST-CyP) fusion protein.

4. The method of claim 1, wherein the **HIV**-1 **Gag** protein is $Pr55^{gag}$.

5. The method of claim 1, wherein the **HIV**-1 **Gag** protein is p24.

6. The method of claim 1, wherein the affinity medium comprises glutathione-agarose beads.

7. The method of claim 1, wherein the amount of said **HIV**-1 **Gag**-CyP affinity fusion protein complex formed is determined using monoclonal antibodies.

8. The method of claim 1, wherein the amount of said **HIV**-1 **Gag**-CyP affinity fusion protein complex formed is determined using polyclonal antibodies.

9. The method of claim 1, wherein the **HIV**-1 **Gag** protein is labeled with a detectable moiety selected from the group consisting of a fluorescent label, a radioactive label, and a chemiluminescent label.

10. The method of claim 1, wherein the **HIV**-1 **Gag**-CyP affinity fusion protein complex is purified and removed from the affinity medium and the amount of **Gag** protein ascertained.

11. A method for identifying compounds capable of interfering with the **formation** of a complex between a cyclophilin (CyP) and an **HIV**-1 **Gag** affinity fusion protein, which comprises the following steps: a) producing an **HIV**-1 **Gag** affinity fusion protein; b) pre-incubating a compound with the **HIV**-1 **Gag** affinity fusion protein of step (a); c) adding a CyP to the incubate of step (b) under conditions which permit the CyP and the **HIV**-1 **Gag** affinity fusion protein to form a complex; d) contacting the incubate of step (c) with an affinity medium under conditions that enable the CyP-**Gag** affinity fusion protein complex to bind to said affinity medium; e) determining the amount of said CyP-**Gag** affinity fusion protein complex **formation** by comparison to a control sample lacking said compound; wherein reduced binding is indicative of the ability of said compound to inhibit CyP-**HIV**-1 **Gag** affinity fusion protein complex **formation**.

12. The method of claim 11, wherein the cyclophilin employed is selected from the group consisting of cyclophilin A, B, C, D, and combinations thereof.

13. The method of claim 11, wherein the **HIV**-1 **Gag** protein employed in the **HIV**-1 **Gag** affinity fusion protein is $Pr55^{gag}$.

14. The method of claim 11, wherein the **HIV**-1 **Gag** protein employed in the **HIV**-1 **Gag** affinity fusion protein is p24.

15. The method of claim 11, wherein the affinity medium comprises glutathione-agarose beads.

16. The method of claim 11, wherein the amount of said CyP-**Gag** affinity fusion protein complex formed is determined using monoclonal antibodies.

17. The method of claim 11, wherein the amount of said CyP-**Gag** affinity fusion protein complex formed is determined using polyclonal antibodies.

18. The method of claim 11, wherein the CyP is labeled with a detectable moiety selected from the group consisting of a fluorescent label, a radioactive label, and a chemiluminescent label.

19. The method of claim 11, wherein the CyP-**HIV**-1 **Gag** affinity fusion protein complex is purified and removed from the affinity medium and the amount of CyP protein ascertained.

20. The method of claim 1, wherein the CyP employed in the CyP affinity fusion protein is CyP A.

21. The method of claim 11, wherein the cyt employed is cyt ...

L25 ANSWER 92 OF 115 USPATFULL on STN
1998:36578 Self assembled defective non-self propagating lentiviral particles.
Mazzara, Gail P., Winchester, MA, United States
Roberts, Bryan, Cambridge, MA, United States
Panicali, Dennis L., Acton, MA, United States
Stallard, Virginia, Seattle, WA, United States
Gritz, Linda R., Somerville, MA, United States
Therion Biologics Corporation, Cambridge, MA, United States (U.S. corporation)
US 5736368 19980407
APPLICATION: US 1995-480779 19950607 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Recombinant viral vectors which coexpress heterologous polypeptides capable of assembling into defective non-self-propagating lentiviral particles are disclosed. The viral vectors as well as the viral particles can be used as immunogens and for targeted delivery of heterologous gene products and drugs.
CLM What is claimed is:
1. An isolated eukaryotic host cell transformed by a pox virus having inserted therein, at least two DNA sequences from a single species of lentivirus DNA sequences wherein one of the lentivirus DNA sequences is selected from the group consisting of the **gag** gene, **gag**-pol and portions thereof, such that the lentivirus DNA sequences express **gag**, **gag**-pol proteins, or portions thereof, referred to as said first lentivirus DNA sequence and a second lentivirus DNA sequence encoding another lentiviral protein, wherein the lentivirus proteins or portions thereof, self-assemble into defective, non-self-propagating lentivirus **particles**.

2. The eukaryotic host cell of claim 1, wherein the pox virus is a vaccinia virus or a fowl pox virus.

3. An isolated eukaryotic host cell transformed by a pox virus vector, said pox virus having inserted therein, at least two DNA sequences from a single species of **human immunodeficiency virus** (**HIV**) wherein one of the **HIV** DNA sequences is selected from the group consisting of the **gag** gene and **gag**- pol gene and said other **HIV** DNA sequence encodes another **HIV** protein, such that the **HIV** DNA sequences either express **gag** proteins, or **gag**-pol proteins, and the **HIV** proteins self-assembled into defective, non-self propagating **HIV particles**.

4. The eukaryotic host cell of claim 3, wherein the pox virus is a vaccinia virus.

5. The eukaryotic host cell of claim 3, wherein said other **HIV** DNA sequence is the env gene.

6. An isolated eukaryotic host cell transformed by a vaccinia virus vector, said vaccinia virus vector comprising a first and a second chimeric gene inserted within the HindIII M region of the vaccinia virus vector, wherein the first chimeric gene comprises an **HIV gag**-pol gene, or a portion thereof, operatively linked to the 40K vaccinia promoter, and the second chimeric gene comprises a different **HIV** gene such that the **gag**, pol proteins, or portions thereof, are co-expressed in the eukaryotic host cell infected with the pox virus vector, and the **gag**, pol and other **HIV** proteins, or portions thereof, self-assemble into defective, non-self-propagating **HIV particles**.

7. The eukaryotic host cell of claim 6, wherein the vaccinia virus vector is selected from the group consisting of vAbT408 and vAbT4674.

96:111363 Chimeric immunogenic gag-V3 virus-like particles of the **human immunodeficiency virus** (**HIV**).
Kang, Chil-Yong, London, Canada
Luo, Lizhong, London, Canada
Korea Green Cross Corporation, Kyongki-Do, Korea, Republic of (non-U.S. corporation)
US 5580773 19961203
APPLICATION: US 1993-100118 19930730 (8) <--
PRIORITY: KR 1992-10493 19920617
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An unprocessed **human immunodeficiency virus** 2 (**HIV**-2) gag precursor protein, containing a deficient protease, assembles into virus-like particles by budding through the cytoplasmic domain of baculovirus-infected cells. Chimeric constructs were generated by coupling the truncated **HIV**-2 gag gene to the neutralizing domain (V3) or the neutralizing and CD4 binding domains (V3+CD4B) of gp120 env gene sequences obtained from **HIV**-1 or **HIV**-2. Virus-like particles were formed by chimeric gene products when the env gene sequences were linked to the 3' terminus of the gag gene. The gag-env chimeric proteins displayed immunoreactivity towards anti-gp120 rabbit antisera.

CLM What is claimed is:

1. A recombinant, chimeric, immunogenic **gag**-env virus-like **particle** of **human immunodeficiency virus** (**HIV**), comprising: (i) an **HIV**-2 **Gag** protein which extends from the amino terminus of **Gag** to a minimum of amino acid 376 and a maximum of amino acid 425, such that said **Gag** protein is capable of forming virus-like **particles**; and, (ii) an **HIV** Env protein linked to the C-terminus of **Gag** containing at least one virus-neutralizing epitope.

2. The recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** according to claim 1, wherein the **gag** coding region includes at least one proline residue at amino acid positions 373, 375, or 377.

3. The recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** according to claim 1, wherein the env gene encodes for a 198 amino acid segment containing the **HIV**-2 V3 loop and CD4 binding domain, and the **gag**-env coding region comprises 574-623 amino acids.

4. The recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** according to claim 1, wherein the env gene encodes for the V3 loop of **HIV** gp120, the CD4-binding domain of **HIV** gp120, or a combination thereof.

5. The recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** according to claim 4, wherein the env gene encodes for a 91 amino acid segment containing the **HIV**-1 gp120 V3 loop; a 90 amino acid segment containing the **HIV**-2 gp120 V3 loop; or a 198 amino acid segment containing the **HIV**-2 V3 loop and CD4-binding domain.

6. The recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** according to claim 1, wherein the env gene encodes for at least two **HIV**-1 gp120 V3 loops placed in tandem.

7. The recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** according to claim 6, wherein at least two of the **HIV**-1 gp120 V3 loops correspond to different viral isolates.

8. A method for the detection of antibodies directed against **human immunodeficiency virus** (**HIV**) in human biological specimens, comprising: (i) immobilizing the recombinant, chimeric, immunogenic **HIV gag**-env virus-like **particle** of claim 1 on a solid support; (ii) contacting said recombinant **particle** with a human biological specimen and allowing immobilized antigen-antibody complex **formation**

immune complexes of step (ii); and (iv) detecting said immune complexes by the addition of a second labeled anti-human antibody.

```
=> file medline
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                        49.08     136.82

FILE 'MEDLINE' ENTERED AT 20:39:11 ON 24 JUN 2004

 FILE LAST UPDATED: 24 JUN 2004 (20040624/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> e trono d/au
E1            3      TRONO A/AU
E2            1      TRONO ANTONIO/AU
E3          105 --> TRONO D/AU
E4            1      TRONO D P/AU
E5            1      TRONO DANIELA/AU
E6           21      TRONO DIDIER/AU
E7            1      TRONO J/AU
E8            2      TRONO K/AU
E9            1      TRONO K G/AU
E10           1      TRONO KARINA/AU
E11           5      TRONO M/AU
E12           1      TRONO M CORAZON/AU

=> s e3 or e4
            105 "TRONO D"/AU
              1 "TRONO D P"/AU
L26         106 "TRONO D"/AU OR "TRONO D P"/AU

=> s l26 and (trans-dominant)
          78085 TRANS
          78391 DOMINANT
            322 TRANS-DOMINANT
                  (TRANS(W)DOMINANT)
L27           1 L26 AND (TRANS-DOMINANT)

=> d l27,cbib,ab,

L27  ANSWER 1 OF 1      MEDLINE on STN
92368716.     PubMed ID: 1503816.    Intracellular immunization:
      trans-dominant mutants of HIV gene products as tools for the study and
      interruption of viral replication. Feinberg M B; Trono D. (Department of
      Medicine, University of California, San Francisco. ) AIDS research and
      human retroviruses, (1992 Jun) 8 (6) 1013-22.  Ref: 134. Journal code:
      8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.


=> d l27,cbib,ab,

L27  ANSWER 1 OF 1      MEDLINE on STN
```

trans-dominant mutants of HIV gene products as tools for the study and interruption of viral replication. Feinberg M B; **Trono D**. (Department of Medicine, University of California, San Francisco.) AIDS research and human retroviruses, (1992 Jun) 8 (6) 1013-22. Ref: 134. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

=> s l26 and (Gag)
11021 GAG
L28 11 L26 AND (GAG)

=> d l28,ti,1-11

L28 ANSWER 1 OF 11 MEDLINE on STN
TI Inhibition of HIV-1 in cell culture by oligonucleotide-loaded nanoparticles.

L28 ANSWER 2 OF 11 MEDLINE on STN
TI A stable system for the high-titer production of multiply attenuated lentiviral vectors.

L28 ANSWER 3 OF 11 MEDLINE on STN
TI A third-generation lentivirus vector with a conditional packaging system.

L28 ANSWER 4 OF 11 MEDLINE on STN
TI Human immunodeficiency virus type 1 matrix protein interacts with cellular protein HO3.

L28 ANSWER 5 OF 11 MEDLINE on STN
TI Human immunodeficiency virus matrix tyrosine phosphorylation: characterization of the kinase and its substrate requirements.

L28 ANSWER 6 OF 11 MEDLINE on STN
TI The Nef protein of human immunodeficiency virus type 1 enhances serine phosphorylation of the viral matrix.

L28 ANSWER 7 OF 11 MEDLINE on STN
TI Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector.

L28 ANSWER 8 OF 11 MEDLINE on STN
TI HIV-1 infection of nondividing cells: C-terminal tyrosine phosphorylation of the viral matrix protein is a key regulator.

L28 ANSWER 9 OF 11 MEDLINE on STN
TI The nuclear localization signal of the matrix protein of human immunodeficiency virus type 1 allows the establishment of infection in macrophages and quiescent T lymphocytes.

L28 ANSWER 10 OF 11 MEDLINE on STN
TI Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells.

L28 ANSWER 11 OF 11 MEDLINE on STN
TI HIV-1 **Gag** mutants can dominantly interfere with the replication of the wild-type virus.

=> d l28,cbib,ab,1-11

L28 ANSWER 1 OF 11 MEDLINE on STN
2001540781. PubMed ID: 11587479. Inhibition of HIV-1 in cell culture by oligonucleotide-loaded nanoparticles. Berton M; Turelli P; **Trono D**; Stein C A; Allemann E; Gurny R. (School of Pharmacy, University of Geneva, Switzerland.. myriam.berton@pharm.unige.ch) . Pharmaceutical research,

country: United States. Language: English.

AB PURPOSE: To investigate the potential use of polymeric nanoparticles for the delivery of antisense oligonucleotides in HIV-1-infected cell cultures. METHODS: Phosphorothioate oligonucleotides were encapsulated into poly (D,L-lactic acid) nanoparticles. Two models of infected cells were used to test the ability of nanoparticles to deliver them. HeLa P4-2 CD4+ cells, stably transfected with the beta-galactosidase reporter gene, were first used to evaluate the activity of the oligonucleotides on a single-round infection cycle. The acutely infected lymphoid CEM cells were then used to evaluate the inhibition of the viral production of HIV-1 by the oligonucleotides. RESULTS: The addition to infected CEM cells of nanoparticles containing **gag** antisense oligonucleotides in the nanomolar range led to strong inhibition of the viral production in a concentration-dependent manner. Similar results were previously observed in HeLa P4-2 CD4+ cells. Nanoparticle-entrapped random-order **gag** oligonucleotides had similar effects on reverse transcription. However, the reverse transcriptase activity of infected cells treated with nanomolar concentrations of free antisense and random oligonucleotides was not affected. CONCLUSIONS: These results suggest that poly (D,L-lactic acid) nanoparticles may have great potential as an efficient delivery system for oligonucleotides in HIV natural target cells, i.e., lymphocytic cells.

L28 ANSWER 2 OF 11 MEDLINE on STN

2000494804. PubMed ID: 10947945. A stable system for the high-titer production of multiply attenuated lentiviral vectors. Klages N; Zufferey R; **Trono D**. (Department of Genetics and Microbiology, University of Geneva, Geneva, Switzerland.) Molecular therapy : journal of the American Society of Gene Therapy, (2000 Aug) 2 (2) 170-6. Journal code: 100890581. ISSN: 1525-0016. Pub. country: United States. Language: English.

AB Lentiviral vectors open exciting perspectives for the genetic treatment of a wide array of inherited and acquired diseases, owing to their ability to govern the efficient delivery, integration, and long-term expression of transgenes into nondividing cells both in vitro and in vivo. The genomic complexity of HIV, where a whole set of genes encode virulence factors essential for pathogenesis but not required for gene transfer, allowed a major step toward clinical acceptability through the creation of multiply attenuated packaging systems. Until now, however, vector particles could only be produced by transient transfection because no high-output, stable packaging cell line was available that produced the latest generation of HIV-based vectors. Here we describe such a line, based on the doxycycline-repressible expression of HIV-1 Rev/**Gag**/Pol and of the vesicular stomatitis virus G envelope (VSV G) in 293 human embryonic kidney cells. Upon induction, the LVG clones can produce 1 to 20 HeLa-transducing units per cell per day for about a week, a yield that compares favorably with that of transiently transfected 293T cells. These virions exhibit functional properties similar to those of viruses produced transiently, in particular the ability to transduce nonmitotic targets. This system will facilitate the further development of lentiviral vectors for gene therapy.

L28 ANSWER 3 OF 11 MEDLINE on STN

1998440501. PubMed ID: 9765382. A third-generation lentivirus vector with a conditional packaging system. Dull T; Zufferey R; Kelly M; Mandel R J; Nguyen M; **Trono D**; Naldini L. (Cell Genesys, Foster City, California 94404, USA.) Journal of virology, (1998 Nov) 72 (11) 8463-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Vectors derived from human immunodeficiency virus (HIV) are highly efficient vehicles for in vivo gene delivery. However, their biosafety is of major concern. Here we exploit the complexity of the HIV genome to provide lentivirus vectors with novel biosafety features. In addition to the structural genes, HIV contains two regulatory genes, tat and rev, that are essential for HIV replication, and four accessory genes that encode critical virulence factors. We previously reported that the HIV type 1

accessory open reading frames are dispensable for efficient gene transduction by a lentivirus vector. We now demonstrate that the requirement for the tat gene can be offset by placing constitutive promoters upstream of the vector transcript. Vectors generated from constructs containing such a chimeric long terminal repeat (LTR) transduced neurons in vivo at very high efficiency, whether or not they were produced in the presence of Tat. When the rev gene was also deleted from the packaging construct, expression of **gag** and pol was strictly dependent on Rev complementation in trans. By the combined use of a separate nonoverlapping Rev expression plasmid and a 5' LTR chimeric transfer construct, we achieved optimal yields of vector of high transducing efficiency (up to 10(7) transducing units [TU]/ml and 10(4) TU/ng of p24). This third-generation lentivirus vector uses only a fractional set of HIV genes: **gag**, pol, and rev. Moreover, the HIV-derived constructs, and any recombinant between them, are contingent on upstream elements and trans complementation for expression and thus are nonfunctional outside of the vector producer cells. This split-genome, conditional packaging system is based on existing viral sequences and acts as a built-in device against the generation of productive recombinants. While the actual biosafety of the vector will ultimately be proven in vivo, the improved design presented here should facilitate testing of lentivirus vectors.

L28 ANSWER 4 OF 11 MEDLINE on STN

1998105821. PubMed ID: 9445076. Human immunodeficiency virus type 1 matrix protein interacts with cellular protein HO3. Lama J; **Trono D.** (Infectious Disease Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA.) Journal of virology, (1998 Feb) 72 (2) 1671-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The matrix (MA) protein of human immunodeficiency virus type 1 (HIV-1) plays a critical role in virion morphogenesis and fulfills important functions during the early steps of infection. In an effort to identify cellular partners of MA, a Saccharomyces cerevisiae two-hybrid screen was utilized. A specific interaction between MA and HO3, a putative histidyl-tRNA synthetase, was demonstrated in this system. HO3-specific mRNA was detected in several tissues relevant for HIV infection, such as spleen, thymus, and peripheral blood lymphocytes, as well as in a number of T-lymphoid-cell lines. The binding of MA to HO3 was confirmed in transfected cells by coimmunoprecipitation. This interaction was abrogated by replacing two lysine residues at positions 26 and 27 of MA by threonine (MA(KK27TT)). HO3 localized both to the cytoplasm and to the nucleus of acutely transfected 293T cells. When overexpressed in HIV-1-producing cells, HO3 was incorporated into wild-type virions but not in ones containing the dilysine-mutated variant of MA. Correspondingly, overexpression of HO3 in virus producer cells enhanced the infectivity of wild-type but not MA(KK27AA) HIV-1 particles. The stimulating effect of HO3 was independent from the presence of Envelope, Vpr, or Vpu. Taken together, these results suggest that HO3, through its recognition of MA, plays a role in the life cycle of HIV-1.

L28 ANSWER 5 OF 11 MEDLINE on STN

97404696. PubMed ID: 9261408. Human immunodeficiency virus matrix tyrosine phosphorylation: characterization of the kinase and its substrate requirements. Camaur D; Gallay P; Swingler S; **Trono D.** (Infectious Disease Laboratory, Salk Institute for Biological Studies, La Jolla, California 92037, USA.) Journal of virology, (1997 Sep) 71 (9) 6834-41. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB During virus assembly, a subset of human immunodeficiency virus (HIV) matrix (MA) molecules is phosphorylated on C-terminal tyrosine. This modification facilitates infection of nondividing cells by allowing for the recruitment of the karyophilic MA into the viral core and preintegration complex. MA tyrosine phosphorylation is accomplished by a cellular protein kinase which is incorporated into virions. In this study, we have investigated the nature of this enzyme as well as the

determinants of its necessary for its phosphorylation. Employing an in vitro kinase assay, we found that the MA tyrosine kinase activity is present in various cultured cell lines including CEM and SupT1 T-lymphoid cells, Namalwa B cells, 293 and CV-1 kidney fibroblasts, and P4 HeLa cells. In addition, it could be detected in platelets, macrophages, and activated peripheral blood lymphocytes (PBLs) but not in erythrocytes and resting PBLs isolated from human blood. Subcellular localization of the kinase activity by cell fractionation demonstrated that it is enriched in cellular membranes. In HIV type 2 (HIV-2) particles, the MA tyrosine kinase is associated with the inner leaflet of the viral membrane, while the tyrosine-phosphorylated MA is localized to the core. Individual mutations of each of the last eight residues immediately upstream of the C-terminal tyrosine (Y132) of HIV-1 MA did not prevent Y132 phosphorylation, suggesting that the kinase does not require a highly specific sequence adjacent to the C-terminal tyrosine. Confirming this, we found that the MA of murine leukemia virus, the sequence of which is only moderately homologous to that of HIV-1 and HIV-2 MA, is also C-terminally tyrosine phosphorylated.

L28 ANSWER 6 OF 11 MEDLINE on STN

97296242. PubMed ID: 9151826. The Nef protein of human immunodeficiency virus type 1 enhances serine phosphorylation of the viral matrix. Swingler S; Gallay P; Camaur D; Song J; Abo A; **Trono D.** (Infectious Disease Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037, USA.) Journal of virology, (1997 Jun) 71 (6) 4372-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 matrix (MA) protein is phosphorylated during virion maturation on its C-terminal tyrosine and on several serine residues. Whereas MA tyrosine phosphorylation facilitates viral nuclear import, the significance of MA serine phosphorylation remains unclear. Here, we report that MA serine but not tyrosine phosphorylation is strongly enhanced by Nef. Mutations that abrogated the membrane association of Nef and its ability to bind a cellular serine/threonine kinase greatly diminished the extent of virion MA serine phosphorylation. Correspondingly, a protein kinase coimmunoprecipitated with Nef could phosphorylate MA on serine in vitro, producing a phosphopeptide pattern reminiscent of that of virion MA. Recombinant p21-activated kinase hPAK65, a recently proposed relative of the Nef-associated kinase, achieved a comparable result. Taken together, these data suggest that MA is a target of the Nef-associated serine kinase.

L28 ANSWER 7 OF 11 MEDLINE on STN

97030203. PubMed ID: 8876144. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Naldini L; Blomer U; Gage F H; **Trono D**; Verma I M. (Salk Institute for Biological Studies, San Diego, CA 92186-5800, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1996 Oct 15) 93 (21) 11382-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We describe the construction of a safe, replication-defective and efficient lentiviral vector suitable for in vivo gene delivery. The reverse transcription of the vector was found to be a rate-limiting step; therefore, promoting the reaction inside the vector particles before delivery significantly enhanced the efficiency of gene transfer. After injection into the brain of adult rats, sustained long-term expression of the transgene was obtained in the absence of detectable pathology. A high proportion of the neurons in the areas surrounding the injection sites of the vector expressed the transduced beta-galactosidase gene. This pattern was invariant in animals sacrificed several months after a single administration of the vector. Transduction occurs by integration of the vector genome, as it was abolished by a single amino acid substitution in the catalytic site of the integrase protein incorporated in the vector. Development of clinically acceptable derivatives of the lentiviral vector may thus enable the sustained delivery of significant amounts of a

[illegible] gene product in a wide variety of somatic tissues.

L28 ANSWER 8 OF 11 MEDLINE on STN

95163091. PubMed ID: 7859280. HIV-1 infection of nondividing cells: C-terminal tyrosine phosphorylation of the viral matrix protein is a key regulator. Gallay P; Swingler S; Aiken C; **Trono D**. (Infectious Disease Laboratory, Salk Institute for Biological Studies, La Jolla, California 92037.) Cell, (1995 Feb 10) 80 (3) 379-88. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB The HIV-1 matrix (MA) protein contains two subcellular localization signals with opposing effects. A myristoylated N-terminus governs particle assembly at the plasma membrane, and a nucleophilic motif facilitates import of the viral preintegration complex into the nucleus of nondividing cells. Here, we show that myristoylation acts as the MA dominant targeting signal in HIV-1 producer cells. During virus assembly, a subset of MA is phosphorylated on the C-terminal tyrosine by a virion-associated cellular protein kinase. Tyrosine-phosphorylated MA is then preferentially transported to the nucleus of target cells. An MA tyrosine mutant virus grows normally in dividing cells, but is blocked for nuclear import in terminally differentiated macrophages. MA tyrosine phosphorylation thus reveals the karyophilic properties of this protein within the HIV-1 preintegration complex, thereby playing a critical role for infection of nondividing cells.

L28 ANSWER 9 OF 11 MEDLINE on STN

94316624. PubMed ID: 8041734. The nuclear localization signal of the matrix protein of human immunodeficiency virus type 1 allows the establishment of infection in macrophages and quiescent T lymphocytes. von Schwedler U; Kornbluth R S; **Trono D**. (Infectious Disease Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037-1099.) Proceedings of the National Academy of Sciences of the United States of America, (1994 Jul 19) 91 (15) 6992-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Lentiviruses, including human immunodeficiency virus type 1 (HIV-1), are unusual among retroviruses in their ability to infect nondividing cells. The matrix proteins of several lentiviruses contain a short stretch of amino acids reminiscent of known nuclear localization signals. In HIV-1, this motif has been shown to function as a nuclear targeting sequence when conjugated to a heterologous protein, and to permit the active nuclear import of the HIV-1 preintegration complex in growth-arrested cells. In the present work, mutations were introduced in the matrix nuclear localization region of T-cell- and macrophage-tropic HIV-1 clones. The resulting viral mutants replicated with normal or even accelerated kinetics in dividing cells, including activated peripheral blood lymphocytes. However, in sharp contrast with wild-type virus, the mutants could not grow efficiently in terminally differentiated macrophages or establish a stable and inducible infection intermediate in unstimulated peripheral blood lymphocytes. Because macrophages represent a major viral reservoir in vivo, and because at any given time most T cells in the body are quiescent, these results strongly suggest that the karyophilic properties of the matrix protein are critical for the spread of the virus in HIV-infected individuals, and consequently for AIDS pathogenesis.

L28 ANSWER 10 OF 11 MEDLINE on STN

93323238. PubMed ID: 8331734. Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. von Schwedler U; Song J; Aiken C; **Trono D**. (Infectious Disease Laboratory, Salk Institute, La Jolla, California 92037-1099.) Journal of virology, (1993 Aug) 67 (8) 4945-55. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) vif gene encodes a 23-kDa protein of unknown function, also produced by most other known lentiviruses. Vif was found to be essential for the spread of HIV-1 in peripheral blood lymphocytes and in primary macrophages, as well as in some but not all established T-cell lines. Vif was required at the stage of viral particle formation, for cell-to-cell as well as for cell-free

transmission of HIV-1. Accordingly, vif-defective viruses could be complemented by the expression of vif in the producer but not in the target cell. vif-defective virions contained wild-type amounts of **Gag** and Env proteins, reverse transcriptase, integrase, genomic RNA, and partial reverse transcripts. Most importantly, they could enter cells normally, and the vif defect could not be rescued through the use of HIV(MLV [murine leukemia virus]) pseudotypes. Instead, vif-mutant viruses were severely impaired in their ability to complete the synthesis of proviral DNA, once internalized in the target cell. These results suggest that Vif plays a role which is novel for a retroviral protein, in allowing the processing and/or the transport of the internalized HIV core.

L28 ANSWER 11 OF 11 MEDLINE on STN
90003221. PubMed ID: 2676192. HIV-1 **Gag** mutants can dominantly interfere with the replication of the wild-type virus. **Trono D**; Feinberg M B; Baltimore D. (Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142.) Cell, (1989 Oct 6) 59 (1) 113-20. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.
AB The products of the human immunodeficiency virus (HIV) **gag** gene exist in a highly multimerized state in the mature virion. For that reason, they may represent a particularly suitable target for the generation of dominant negative mutants. A number of HIV site-directed **Gag** mutants did show interference with the production of infectious viral particles from cells in which they were cotransfected with a wild-type proviral DNA. Furthermore, cells constitutively expressing such HIV **Gag** mutants had an impaired ability to support HIV replication when infected with wild-type virus. The block was localized to the late stages of the virus life cycle. Such **Gag** variants could constitute prototypes for the development of anti-HIV intracellular immunization.

=> d his

(FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
E PREVELIGE P/AU

FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1 1 S US5716613/PN
L2 1 S US5789245/PN

FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
E PREVELIGE P E/AU
L3 35 S E3 OR E4 OR E5
E KLISHKO V Y/AU
L4 4 S E2-E4
E GROSS I/AU
E HOHENBERG H/AU
L5 39 S E3 OR E4
E CAMPBELL S/AU
L6 864 S E3
E REIN A/AU
L7 97 S E3
L8 4 S L6 AND L7
E EHRLICH L S/AU
L9 13 S E3
E KLIKOVA M/AU
L10 2 S E3
E SAKALIAN M/AU
L11 14 S E3 OR E4
E SMITH A J/AU
L12 594 S E3
L13 17 S L12 AND PY=1990
L14 1 S L13 AND ASSEMBLED
E LEIS J/AU

```
L16             8 S L15 AND PY=1988

     FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
L17         33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L18         23040 S L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOC
L19            66 S L18 AND (IN VITRO ASSEMBLY)
L20            66 S L19 AND (ASSEMBLY OR MORPHOGENESIS OR FORMATION OR VIRUS-LIKE
L21            35 S L20 AND AY<2001
L22          2371 S L17 AND (GAG/CLM OR CA/CLM OR MA/CLM OR NC/CLM OR CAPSID/CLM
L23           664 S L22 AND (ASSEMBLY/CLM OR PARTICLE?/CLM OR MORPHOGENESIS/CLM O
L24           248 S L23 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L25           115 S L24 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 20:39:11 ON 24 JUN 2004
                E TRONO D/AU
L26           106 S E3 OR E4
L27             1 S L26 AND (TRANS-DOMINANT)
L28            11 S L26 AND (GAG)

=> s (HIV or human immunodeficiency virus)
           137763 HIV
          8566983 HUMAN
           113554 IMMUNODEFICIENCY
           376454 VIRUS
            43100 HUMAN IMMUNODEFICIENCY VIRUS
                  (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L29        142537 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l29 (Gag or CA or NC or MA or capsid or nucleocapsid or matrix)
MISSING OPERATOR 'L29 (GAG'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l29 and (gag or MA or CA or NC or matrix or capsid or nucleocapsid)
            11021 GAG
            50402 MA
           111157 CA
             5655 NC
           113887 MATRIX
            14811 CAPSID
             4029 NUCLEOCAPSID
L30          6071 L29 AND (GAG OR MA OR CA OR NC OR MATRIX OR CAPSID OR NUCLEOCAPS
                  ID)

=> s l30 and (in vitro assembly)
           765570 VITRO
            42077 ASSEMBLY
              460 IN VITRO ASSEMBLY
                    (VITRO(W)ASSEMBLY)
L31            12 L30 AND (IN VITRO ASSEMBLY)

=> d l31,cbib,ab,1-12

L31  ANSWER 1 OF 12     MEDLINE on STN
2003592094.     PubMed ID: 14672655.   Interaction with capsid protein alters
     RNA structure and the pathway for in vitro assembly of cowpea
     chlorotic mottle virus. Johnson Jennifer M; Willits Deborah A; Young Mark
     J; Zlotnick Adam. (Department of Biochemistry and Molecular Biology,
     University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190,
     USA. ) Journal of molecular biology, (2004 Jan 9) 335 (2) 455-64.  Journal
     code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom.
     Language: English.
AB   Viruses use sophisticated mechanisms to allow the specific packaging of
     their genome over that of host nucleic acids.  We examined the in vitro
     assembly of the Cowpea chlorotic mottle virus (CCMV) and observed that
```

assembly with viral RNA follows one of three mechanisms. Briefly, 180 **capsid** protein (CP) dimers bind RNA with low cooperativity and form virus-like particles of 90 CP dimers and one copy of RNA. Longer incubation reveals a different assembly path. At a stoichiometry of about ten CP dimers per RNA, the CP slowly folds the RNA into a compact structure that can be bound with high cooperativity by additional CP dimers. This folding process is exclusively a function of CP quaternary structure and is independent of RNA sequence. CP-induced folding is distinct from RNA folding that depends on base-pairing to stabilize tertiary structure. We hypothesize that specific encapsidation of viral RNA is a three-step process: specific binding by a few copies of CP, RNA folding, and then cooperative binding of CP to the "labeled" nucleoprotein complex. This mechanism, observed in a plant virus, may be applicable to other viruses that do not halt synthesis of host nucleic acid, including **HIV**.

L31 ANSWER 2 OF 12 MEDLINE on STN

2002630683. PubMed ID: 12388738. Reversible binding of recombinant **human immunodeficiency virus** type 1 **gag** protein to nucleic acids in virus-like particle assembly in vitro. Feng Ya-Xiong; Li Tong; Campbell Stephen; Rein Alan. (HIV Drug Resistance Program, National Cancer Institute-Frederick, Frederick, Maryland 21702-1201, USA.) Journal of virology, (2002 Nov) 76 (22) 11757-62. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Recombinant **human immunodeficiency virus** type 1 (**HIV**-1) **Gag** protein can assemble into virus-like particles (VLPs) in suitable buffer conditions with nucleic acid. We have explored the role of nucleic acid in this assembly process. **HIV**-1 **nucleocapsid** protein, a domain of **Gag**, can bind to oligodeoxynucleotides with the sequence d(TG)(n) with more salt resistance than to d(A)(n) oligonucleotides. We found that assembly of VLPs on d(TG)(n) oligonucleotides was more salt resistant than assembly on d(A)(n); thus, the oligonucleotides do not simply neutralize basic residues in **Gag** but provide a binding surface upon which **Gag** molecules assemble into VLPs. We also found that **Gag** molecules could be "trapped" on internal d(TG)(n) sequences within 40-base oligonucleotides, rendering them unable to take part in assembly. Thus, assembly on oligonucleotides requires that **Gag** proteins bind near the ends of the nucleic acid, and binding of **Gag** to internal d(TG)(n) sequences is apparently cooperative. Finally, we showed that nucleic acids in VLPs can exchange with nucleic acids in solution; there is a hierarchy of preferences in these exchange reactions. The results are consistent with an equilibrium model of in **vitro assembly** and may help to explain how **Gag** molecules in vivo select genomic RNA despite the presence in the cell of a vast excess of cellular mRNA molecules.

L31 ANSWER 3 OF 12 MEDLINE on STN

2002611458. PubMed ID: 12368324. The Mason-Pfizer monkey virus internal scaffold domain enables in **vitro assembly** of **human immunodeficiency virus** type 1 **Gag**. Sakalian Michael; Dittmer Stephanie S; Gandy A Dustin; Rapp Nathan D; Zabransky Ales; Hunter Eric. (Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190, USA.. mike-sakalian@ouhsc.edu) . Journal of virology, (2002 Nov) 76 (21) 10811-20. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The Mason-Pfizer monkey virus (M-PMV) **Gag** protein possesses the ability to assemble into an immature **capsid** when synthesized in a reticulocyte lysate translation system. In contrast, the **human immunodeficiency virus** (**HIV**) **Gag** protein is incapable of assembly in parallel assays. To enable the assembly of **HIV Gag**, we have combined or inserted regions of M-PMV **Gag** into **HIV Gag**. By both biochemical and morphological criteria, several of these chimeric **Gag** molecules are capable of assembly into immature **capsid**-like structures in this in vitro system. Chimeric species containing large regions of M-PMV **Gag** fused to **HIV Gag** sequences failed to assemble, while species consisting of only the M-PMV p12 region, and its internal scaffold domain

(ISD), fused to **HIV Gag** were capable of assembly, albeit at reduced kinetics compared to M-PMV **Gag**. The ability of the ISD to induce assembly of **HIV Gag**, which normally assembles at the plasma membrane, suggests a common requirement for a concentrating factor in retrovirus assembly. Despite the dramatic effect of the ISD on chimera assembly, the function of **HIV Gag** domains in that process was found to remain essential, since an assembly-defective mutant of **HIV CA**, M185A, abolished assembly when introduced into the chimera. This continued requirement for **HIV Gag** domain function in the assembly of chimeric molecules will allow this in vitro system to be used for the analysis of potential inhibitors of **HIV** immature particle assembly.

L31 ANSWER 4 OF 12 MEDLINE on STN

2001443061. PubMed ID: 11488604. In **vitro assembly** of feline immunodeficiency virus **capsid** protein: biological role of conserved cysteines. Nath M D; Peterson D L. (National Cancer Institute, NIH, Frederick, Maryland 21702, USA.. nathm@ncifcrf.gov) . Archives of biochemistry and biophysics, (2001 Aug 15) 392 (2) 287-94. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB Core assembly, a key step in the retroviral life cycle, is poorly understood. Previous studies have shown that the entire **gag** region is needed to form the assembled particles. In this report, we have shown that the assembly process is driven by recombinant **capsid** protein (p26) of feline immunodeficiency virus itself. Proteins are expressed in a bacterial system and soluble forms of wild-type and modified proteins are purified from bacterial extracts and are examined on gel-filtration chromatography fitted to an HPLC system. It has also been shown that changing residue Cys190 (one of the two conserved cysteines of feline immunodeficiency virus which are also conserved for all the immunodeficiency viruses including **HIV**) to serine by site-directed mutagenesis disrupts the assembly process. In addition, this modification causes considerable thermal instability of the protein while substitutions at nonconserved cysteines do not significantly affect the thermal stability and assembly of the protein. These findings indicate that conserved cysteine residues play a vital role in the **capsid** protein assembly and, therefore, are critical for virus infectivity.

L31 ANSWER 5 OF 12 MEDLINE on STN

2001092665. PubMed ID: 11134289. Organization of immature **human immunodeficiency virus** type 1. Wilk T; Gross I; Gowen B E; Rutten T; de Haas F; Welker R; Krausslich H G; Boulanger P; Fuller S D. (The Structural Biology Programme, European Molecular Biology Laboratory, D69012 Heidelberg, Federal Republic of Germany.) Journal of virology, (2001 Jan) 75 (2) 759-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Immature retrovirus particles contain radially arranged **Gag** polyproteins in which the N termini lie at the membrane and the C termini extend toward the particle's center. We related image features to the polyprotein domain structure by combining mutagenesis with cryoelectron microscopy and image analysis. The **matrix** (**MA**) domain appears as a thin layer tightly associated with the inner face of the viral membrane, separated from the **capsid** (**CA**) layer by a low-density region corresponding to its C terminus. Deletion of the entire p6 domain has no effect on the width or spacing of the density layers, suggesting that p6 is not ordered in immature **human immunodeficiency virus** type 1 (**HIV**-1). In **vitro assembly** of a recombinant **Gag** polyprotein containing only **capsid** (**CA**) and **nucleocapsid** (**NC**) domains results in the formation of nonenveloped spherical particles which display two layers with density matching that of the **CA-NC** portion of immature **HIV**-1 **Gag** particles. Authentic, immature **HIV**-1 displays additional surface features and an increased density between the lipid bilayers which reflect the presence of gp41. The other internal features match those of virus-like particles.

L31 ANSWER 6 OF 12 MEDLINE on STN

2000091966. PubMed ID: 10684257. Biochemical and structural analysis of isolated mature cores of **human immunodeficiency virus** type 1. Welker R; Hohenberg H; Tessmer U; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, D-20251 Hamburg, Germany.) Journal of virology, (2000 Feb) 74 (3) 1168-77. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mature **human immunodeficiency virus** type 1 (**HIV**-1) particles contain a cone-shaped core structure consisting of the internal ribonucleoprotein complex encased in a proteinaceous shell derived from the viral **capsid** protein. Because of their very low stability after membrane removal, **HIV**-1 cores have not been purified in quantities sufficient for structural and biochemical analysis. Based on our in **vitro assembly** experiments, we have developed a novel method for isolation of intact mature **HIV**-1 cores. Concentrated virus suspensions were briefly treated with nonionic detergent and immediately centrifuged in a microcentrifuge for short periods of time. The resuspended pellet was subsequently analyzed by negative-stain and thin-section electron microscopy and by immunoelectron microscopy. Abundant cone-shaped cores as well as tubular and aberrant structures were observed. Stereo images showed that core structures preserved their three-dimensional architecture and exhibited a regular substructure. Detailed analysis of 155 cores revealed an average length of **ca**. 103 nm, an average diameter at the base of **ca**. 52 nm, and an average angle of 21.3 degrees. There was significant variability in all parameters, indicating that **HIV** cores are not homogeneous. Immunoblot analysis of core preparations allowed semiquantitative estimation of the relative amounts of viral and cellular proteins inside the **HIV**-1 core, yielding a model for the topology of various proteins inside the virion.

L31 ANSWER 7 OF 12 MEDLINE on STN

2000086825. PubMed ID: 10619849. A conformational switch controlling **HIV**-1 morphogenesis. Gross I; Hohenberg H; Wilk T; Wiegers K; Grattinger M; Muller B; Fuller S; Krausslich H G. (Heinrich-Pette-Institut fur experimentelle Virologie und Immunologie an der Universitat Hamburg, Martinistrasse 52, D-20251 Hamburg, Germany.) EMBO journal, (2000 Jan 4) 19 (1) 103-13. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Assembly of infectious **human immunodeficiency virus** type 1 (**HIV**-1) proceeds in two steps. Initially, an immature virus with a spherical **capsid** shell consisting of uncleaved **Gag** polyproteins is formed. Extracellular proteolytic maturation causes rearrangement of the inner virion structure, leading to the conical **capsid** of the infectious virus. Using an in **vitro assembly** system, we show that the same **HIV**-1 **Gag**-derived protein can form spherical particles, virtually indistinguishable from immature **HIV**-1 capsids, as well as tubular or conical particles, resembling the mature core. The assembly phenotype could be correlated with differential binding of the protein to monoclonal antibodies recognizing epitopes in the **HIV**-1 **capsid** protein (**CA**), suggesting distinct conformations of this domain. Only tubular and conical particles were observed when the protein lacked spacer peptide SP1 at the C-terminus of **CA**, indicating that SP1 may act as a molecular switch, whose presence determines spherical **capsid** formation, while its cleavage leads to maturation.

L31 ANSWER 8 OF 12 MEDLINE on STN

1999419092. PubMed ID: 10488150. In **vitro assembly** of **human immunodeficiency virus** type 1 **Gag** protein. Morikawa Y; Goto T; Sano K. (The Kitasato Institute, Shirokane 5-9-1, Minato-ku, Tokyo 108-8642, Japan.. ymorikawa@kitasato.or.jp) . Journal of biological chemistry, (1999 Sep 24) 274 (39) 27997-8002. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Retroviral **Gag** protein is sufficient to produce **Gag** virus-like particles when expressed in higher eukaryotic cells. Here we describe the in **vitro assembly** reaction of **human immunodeficiency virus Gag** protein, which consists of two sequential steps showing the optimal

conditions for each reaction. Following expression and purification, **Gag** protein lacking only the C-terminal p6 domain was present as a monomer (50 kDa) by velocity sedimentation analysis. Initial assembly of the **Gag** protein to 60 S intermediates occurred by dialysis at 4 degrees C in low salt at neutral to alkaline pH. However, higher order of assembly required incubation at 37 degrees C and was facilitated by the addition of Mg(2+). Prolonged incubation under these conditions produced complete assembly (600 S), equivalent to **Gag** virus-like particles obtained from **Gag**-expressing cells. Neither form disassembled by treatment with nonionic detergent, suggesting that correct assembly might occur in vitro. Electron microscopic observation confirmed that the 600 S assembly products were spherical particles similar to authentic immature **human immunodeficiency virus** particles. The latter assembly stage but not the former was accelerated by the addition of RNA although not inhibited by RNaseA treatment. These results suggest that **Gag** protein alone assembles in vitro, but that additional RNA facilitates the assembly reaction.

L31 ANSWER 9 OF 12 MEDLINE on STN

1999225664. PubMed ID: 10208938. In **vitro assembly** properties of wild-type and cyclophilin-binding defective **human immunodeficiency virus capsid** proteins in the presence and absence of cyclophilin A. Grattinger M; Hohenberg H; Thomas D; Wilk T; Muller B; Krausslich H G. (Heinrich-Pette-Institut fur Experimentelle Virologie und Immunologie an der, Universitat Hamburg, Hamburg, D-20251, Germany.) Virology, (1999 Apr 25) 257 (1) 247-60. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The cellular protein cyclophilin A (CypA) binds specifically to the **human immunodeficiency virus** type 1 (**HIV**-1) **capsid** (**CA**) protein and is incorporated into **HIV**-1 particles at a molar ratio of 1:10 (CypA/**CA**). Structural analysis of a **CA**-CypA complex suggested that CypA may destabilize interactions in the viral **capsid** and thus promote uncoating. We analyzed the influence of CypA on the in **vitro assembly** properties of wild-type (WT) **CA** and derivatives containing substitutions of Gly89 in the Cyp-binding loop. All variant proteins were significantly impaired in CypA binding. In the presence of CypA at a molar ratio of 1:10 (CypA/**CA**), WT **CA** assembled into hollow cylinders that were similar to those observed in the absence of CypA but slightly longer. Higher CypA concentrations inhibited cylinder formation. Variant **CA** proteins G89L and G89F yielded similar cylinders as the WT protein but were significantly more resistant to CypA. Cryoelectron microscopic analysis of WT cylinders assembled in the presence of CypA revealed direct binding of CypA to the outer surface. Electron diffraction patterns generated from these cylinders indicated that CypA causes local disorder. The addition of CypA to preassembled cylinders had little effect, however, and cylinders were only disrupted when incubated with a threefold molar excess of CypA for several hours. These results suggest that CypA does not efficiently destabilize **CA** interactions at the molar ratio observed in the virion and therefore is unlikely to serve as an uncoating factor. Copyright 1999 Academic Press.

L31 ANSWER 10 OF 12 MEDLINE on STN

1999139010. PubMed ID: 9971810. In **vitro assembly** properties of **human immunodeficiency virus** type 1 **Gag** protein lacking the p6 domain. Campbell S; Rein A. (ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702, USA.. campbells@mail.ncifcrf.gov) . Journal of virology, (1999 Mar) 73 (3) 2270-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) normally assembles into particles of 100 to 120 nm in diameter by budding through the plasma membrane of the cell. The **Gag** polyprotein is the only viral protein that is required for the formation of these particles. We have used an in **vitro assembly** system to examine the assembly properties of purified, recombinant **HIV**-1 **Gag** protein and of **Gag** missing the C-terminal p6 domain (**Gag** Deltap6). This system was used previously to show that the

CA-NC fragment of HIV-1 Gag assembled into cylindrical particles. We now report that both **HIV**-1 **Gag** and **Gag** Deltap6 assemble into small, 25- to 30-nm-diameter spherical particles in vitro. The multimerization of **Gag** Deltap6 into units larger than dimers and the formation of spherical particles required nucleic acid. Removal of the nucleic acid with NaCl or nucleases resulted in the disruption of the multimerized complexes. We conclude from these results that (i) N-terminal extension of **HIV**-1 **CA-NC** to include the **MA** domain results in the formation of spherical, rather than cylindrical, particles; (ii) nucleic acid is required for the assembly and maintenance of **HIV**-1 **Gag** Deltap6 virus-like particles in vitro and possibly in vivo; (iii) a wide variety of RNAs or even short DNA oligonucleotides will support assembly; (iv) protein-protein interactions within the particle must be relatively weak; and (v) recombinant **HIV**-1 **Gag** Deltap6 and nucleic acid are not sufficient for the formation of normal-sized particles.

L31 ANSWER 11 OF 12 MEDLINE on STN

1998241716. PubMed ID: 9573245. N-Terminal extension of **human immunodeficiency virus capsid** protein converts the in **vitro assembly** phenotype from tubular to spherical particles. Gross I; Hohenberg H; Huckhagel C; Krausslich H G. (Heinrich-Pette-Institut, D-20251 Hamburg, Germany.) Journal of virology, (1998 Jun) 72 (6) 4798-810. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Expression of retroviral **Gag** polyproteins is sufficient for morphogenesis of virus-like particles with a spherical immature protein shell. Proteolytic cleavage of **Gag** into the **matrix** (**MA**), **capsid** (**CA**), **nucleocapsid** (**NC**), and p6 domains (in the case of **human immunodeficiency virus** [**HIV**]) leads to condensation to the mature cone-shaped core. We have analyzed the formation of spherical or cylindrical particles on in **vitro assembly** of purified **HIV** proteins or inside Escherichia coli cells. **CA** protein alone yielded cylindrical particles, while all N-terminal extensions of **CA** abolished cylinder formation. Spherical particles with heterogeneous diameters or amorphous protein aggregates were observed instead. Extending **CA** by 5 amino acids was sufficient to convert the assembly phenotype to spherical particles. Sequences C-terminal of **CA** were not required for sphere formation. Proteolytic cleavage of N-terminally extended **CA** proteins prior to in **vitro assembly** led to the formation of cylindrical particles, while proteolysis of in **vitro assembly** products caused disruption of spheres but not formation of cylinders. In **vitro assembly** of **CA** and extended **CA** proteins in the presence of cyclophilin A (CypA) at a **CA**-to-CypA molar ratio of 10:1 yielded significantly longer cylinders and heterogeneous spheres, while higher concentrations of CypA completely disrupted particle formation. We conclude that the spherical shape of immature **HIV** particles is determined by the presence of an N-terminal extension on the **CA** domain and that core condensation during virion maturation requires the liberation of the N terminus of **CA**.

L31 ANSWER 12 OF 12 MEDLINE on STN

1998036138. PubMed ID: 9370371. In **vitro assembly** properties of purified bacterially expressed **capsid** proteins of **human immunodeficiency virus**. Gross I; Hohenberg H; Krausslich H G. (Heinrich-Pette-Institut, Hamburg, Germany.) European journal of biochemistry / FEBS, (1997 Oct 15) 249 (2) 592-600. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The **Gag** polyprotein of retroviruses is sufficient for assembly and budding of virus-like particles from the host cell. In the case of **human immunodeficiency virus** (**HIV**), **Gag** contains the domains **matrix**, **capsid** (**CA**), **nucleocapsid** (**NC**) and p6 which are separated by the viral proteinase inside the nascent virion, leading to morphological maturation to yield an infectious virus. In the mature virus, **CA** forms a **capsid** shell surrounding the ribonucleoprotein core consisting of **NC** and the genomic RNA. To define requirements for particle assembly and functional contributions of individual domains, we

expressed domains of **HIV Gag** in Escherichia coli and purified the products to near homogeneity. In **vitro assembly** of **CA**, with or without the C-terminally adjacent spacer peptide, yielded tubular structures with a diameter of approximately 55 nm and heterogeneous length. Efficient particle formation required high protein concentration, high salt and neutral to alkaline pH. In contrast, in **vitro assembly** of **CA-NC** occurred at a 20-fold lower protein concentration and in low salt, but required addition of RNA. These results suggest that hydrophobic interactions of **capsid** proteins are sufficient for particle formation while the RNA-binding **nucleocapsid** domain may concentrate and align structural proteins on the viral genome.

```
=> d his

     (FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

     FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
                E PREVELIGE P/AU

     FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1              1 S US5716613/PN
L2              1 S US5789245/PN

     FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
                E PREVELIGE P E/AU
L3             35 S E3 OR E4 OR E5
                E KLISHKO V Y/AU
L4              4 S E2-E4
                E GROSS I/AU
                E HOHENBERG H/AU
L5             39 S E3 OR E4
                E CAMPBELL S/AU
L6            864 S E3
                E REIN A/AU
L7             97 S E3
L8              4 S L6 AND L7
                E EHRLICH L S/AU
L9             13 S E3
                E KLIKOVA M/AU
L10             2 S E3
                E SAKALIAN M/AU
L11            14 S E3 OR E4
                E SMITH A J/AU
L12           594 S E3
L13            17 S L12 AND PY=1990
L14             1 S L13 AND ASSEMBLED
                E LEIS J/AU
L15            65 S E3
L16             8 S L15 AND PY=1988

     FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
L17         33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L18         23040 S L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOC
L19            66 S L18 AND (IN VITRO ASSEMBLY)
L20            66 S L19 AND (ASSEMBLY OR MORPHOGENESIS OR FORMATION OR VIRUS-LIKE
L21            35 S L20 AND AY<2001
L22          2371 S L17 AND (GAG/CLM OR CA/CLM OR MA/CLM OR NC/CLM OR CAPSID/CLM
L23           664 S L22 AND (ASSEMBLY/CLM OR PARTICLE?/CLM OR MORPHOGENESIS/CLM O
L24           248 S L23 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L25           115 S L24 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 20:39:11 ON 24 JUN 2004
                E TRONO D/AU
L26           106 S E3 OR E4
L27             1 S L26 AND (TRANS-DOMINANT)
```

```
L29      142537 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L30        6071 S L29 AND (GAG OR MA OR CA OR NC OR MATRIX OR CAPSID OR NUCLEOC
L31          12 S L30 AND (IN VITRO ASSEMBLY)

=> s l30 and (inhibitor? or antiviral?)
        705765 INHIBITOR?
         39971 ANTIVIRAL?
L32       1088 L30 AND (INHIBITOR? OR ANTIVIRAL?)

=> s l32 and (small molecule)
        505656 SMALL
        119962 MOLECULE
          3002 SMALL MOLECULE
                 (SMALL(W)MOLECULE)
L33          6 L32 AND (SMALL MOLECULE)

=> d l33,ti,1-6

L33  ANSWER 1 OF 6     MEDLINE on STN
TI   Small-molecule inhibition of human immunodeficiency virus type 1
     replication by specific targeting of the final step of virion maturation.

L33  ANSWER 2 OF 6     MEDLINE on STN
TI   Further studies on 2,4-diamino-5-(2',5'-disubstituted benzyl)pyrimidines
     as potent and selective inhibitors of dihydrofolate reductases from
     three major opportunistic pathogens of AIDS.

L33  ANSWER 3 OF 6     MEDLINE on STN
TI   Anticancer and antiviral sulfonamides.

L33  ANSWER 4 OF 6     MEDLINE on STN
TI   Association of chemokine-mediated block to HIV entry with coreceptor
     internalization.

L33  ANSWER 5 OF 6     MEDLINE on STN
TI   Identification of potent, selective non-peptide CC chemokine receptor-3
     antagonist that inhibits eotaxin-, eotaxin-2-, and monocyte chemotactic
     protein-4-induced eosinophil migration.

L33  ANSWER 6 OF 6     MEDLINE on STN
TI   Ribozyme mimics as catalytic antisense reagents.


=> d l33,cbib,ab,1

L33  ANSWER 1 OF 6     MEDLINE on STN
2004019674.    PubMed ID: 14694123.   Small-molecule inhibition of human
     immunodeficiency virus type 1 replication by specific targeting of the
     final step of virion maturation. Zhou Jing; Yuan Xiong; Dismuke David;
     Forshey Brett M; Lundquist Christopher; Lee Kuo-Hsiung; Aiken Christopher;
     Chen Chin Ho. (Department of Microbiology and Immunology, Vanderbilt
     University School of Medicine, Nashville, Tennessee 37232, USA. ) Journal
     of virology, (2004 Jan) 78 (2) 922-9.  Journal code: 0113724. ISSN:
     0022-538X. Pub. country: United States. Language: English.
AB   Despite the effectiveness of currently available human
     immunodeficiency virus type 1 (HIV-1) therapies, a continuing need
     exists for new drugs to treat HIV-1 infection.  We investigated the
     mechanism by which 3-O-[3',3'-dimethylsuccinyl]-betulinic acid (DSB)
     inhibits HIV-1 replication.  DSB functions at a late stage of the virus
     life cycle but does not inhibit the HIV-1 protease in vitro or interfere
     with virus assembly or release.  DSB specifically delays the cleavage of
     Gag between the capsid (CA) and p2, resulting in delayed formation
     of the mature viral core and reduced HIV-1 infectivity.  Replication of
     simian immunodeficiency virus (SIV) was resistant to DSB; however, a
     chimeric SIV carrying CA-p2 sequences from HIV-1 was inhibited by the
```

drug, indicating that susceptibility to DSB maps to the **CA**-p2 region of the **HIV**-1 **Gag** protein. A single point mutation at the **CA**-p2 cleavage site of **HIV**-1 conferred strong resistance to DSB, confirming the target of the drug. **HIV**-1 strains that are resistant to a variety of protease **inhibitors** were sensitive to DSB. These findings indicate that DSB specifically protects the **CA**-p2 cleavage site from processing by the viral protease during virion maturation, thereby revealing a novel mechanism for pharmacologic inhibition of **HIV**-1 replication.

=> d his

(FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
E PREVELIGE P/AU

FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1 1 S US5716613/PN
L2 1 S US5789245/PN

FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
E PREVELIGE P E/AU
L3 35 S E3 OR E4 OR E5
E KLISHKO V Y/AU
L4 4 S E2-E4
E GROSS I/AU
E HOHENBERG H/AU
L5 39 S E3 OR E4
E CAMPBELL S/AU
L6 864 S E3
E REIN A/AU
L7 97 S E3
L8 4 S L6 AND L7
E EHRLICH L S/AU
L9 13 S E3
E KLIKOVA M/AU
L10 2 S E3
E SAKALIAN M/AU
L11 14 S E3 OR E4
E SMITH A J/AU
L12 594 S E3
L13 17 S L12 AND PY=1990
L14 1 S L13 AND ASSEMBLED
E LEIS J/AU
L15 65 S E3
L16 8 S L15 AND PY=1988

FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
L17 33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L18 23040 S L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOC
L19 66 S L18 AND (IN VITRO ASSEMBLY)
L20 66 S L19 AND (ASSEMBLY OR MORPHOGENESIS OR FORMATION OR VIRUS-LIKE
L21 35 S L20 AND AY<2001
L22 2371 S L17 AND (GAG/CLM OR CA/CLM OR MA/CLM OR NC/CLM OR CAPSID/CLM
L23 664 S L22 AND (ASSEMBLY/CLM OR PARTICLE?/CLM OR MORPHOGENESIS/CLM O
L24 248 S L23 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L25 115 S L24 AND AY<2001

FILE 'MEDLINE' ENTERED AT 20:39:11 ON 24 JUN 2004
E TRONO D/AU
L26 106 S E3 OR E4
L27 1 S L26 AND (TRANS-DOMINANT)
L28 11 S L26 AND (GAG)
L29 142537 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L30 6071 S L29 AND (GAG OR MA OR CA OR NC OR MATRIX OR CAPSID OR NUCLEOC

L32 1088 S L30 AND (INHIBITOR? OR ANTIVIRAL?)
L33 6 S L32 AND (SMALL MOLECULE)

=> s l32 and (Gag inhibitor? or capsid inhibitor? or matrix inhibitor? or nucleocapsid inhibito
11021 GAG
705765 INHIBITOR?
0 GAG INHIBITOR?
(GAG(W)INHIBITOR?)
14811 CAPSID
705765 INHIBITOR?
1 CAPSID INHIBITOR?
(CAPSID(W)INHIBITOR?)
113887 MATRIX
705765 INHIBITOR?
2 MATRIX INHIBITOR?
(MATRIX(W)INHIBITOR?)
4029 NUCLEOCAPSID
705765 INHIBITOR?
3 NUCLEOCAPSID INHIBITOR?
(NUCLEOCAPSID(W)INHIBITOR?)
L34 3 L32 AND (GAG INHIBITOR? OR CAPSID INHIBITOR? OR MATRIX INHIBITOR
? OR NUCLEOCAPSID INHIBITOR?)

=> d l34,cbib,1-3

L34 ANSWER 1 OF 3 MEDLINE on STN
2001408982. PubMed ID: 11248362. Inhibition of the early phase of **HIV** replication by an isothiazolone, PD 161374. Sharmeen L; McQuade T; Heldsinger A; Gogliotti R; Domagala J; Gracheck S. (Department of Infectious Diseases, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, USA.) Antiviral research, (2001 Feb) 49 (2) 101-14. Journal code: 8109699. ISSN: 0166-3542. Pub. country: Netherlands. Language: English.

L34 ANSWER 2 OF 3 MEDLINE on STN
2000027225. PubMed ID: 10559314. Multiple effects of an anti-**human immunodeficiency virus nucleocapsid inhibitor** on virus morphology and replication. Berthoux L; Pechoux C; Darlix J L. (LaboRetro, Unite de Virologie Humaine INSERM-ENS no. 412, Ecole Normale Superieure, 69364 Lyon Cedex 07, France.) Journal of virology, (1999 Dec) 73 (12) 10000-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

L34 ANSWER 3 OF 3 MEDLINE on STN
97208923. PubMed ID: 9055865. Azodicarbonamide inhibits **HIV**-1 replication by targeting the **nucleocapsid** protein. Rice W G; Turpin J A; Huang M; Clanton D; Buckheit R W Jr; Covell D G; Wallqvist A; McDonnell N B; DeGuzman R N; Summers M F; Zalkow L; Bader J P; Haugwitz R D; Sausville E A. (Laboratory of Antiviral Drug Mechanisms, National Cancer Institute-Frederick Cancer Research and Development Center, SAIC Frederick, Maryland 21702, USA.) Nature medicine, (1997 Mar) 3 (3) 341-5. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

=> d l34,cbib,ab,2,3

L34 ANSWER 2 OF 3 MEDLINE on STN
2000027225. PubMed ID: 10559314. Multiple effects of an anti-**human immunodeficiency virus nucleocapsid inhibitor** on virus morphology and replication. Berthoux L; Pechoux C; Darlix J L. (LaboRetro, Unite de Virologie Humaine INSERM-ENS no. 412, Ecole Normale Superieure, 69364 Lyon Cedex 07, France.) Journal of virology, (1999 Dec) 73 (12) 10000-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

major structural component of the virion core and a key factor involved in proviral DNA synthesis and virus formation. 2,2'-Dithiobenzamides (DIBA-1) and related compounds that are **inhibitors** of NCp7 are thought to eject zinc ions from NCp7 zinc fingers, inhibiting the maturation of virion proteins. Here, we show that the presence of DIBA-1 at the time of virus formation causes morphological malformations of the virus and reduces proviral DNA synthesis. Thus, it seems that DIBA-1 is responsible for a "core-freezing effect," as shown by electron microscopy analyses. DIBA-1 can also directly interfere with the fate of the newly made proviral DNA in a manner independent of its effects on virion core formation. These data strongly suggest that **nucleocapsid** protein is a prime target for new compounds aimed at inhibiting **human immunodeficiency virus** and other retroviruses.

L34 ANSWER 3 OF 3 MEDLINE on STN
97208923. PubMed ID: 9055865. Azodicarbonamide inhibits **HIV**-1 replication by targeting the **nucleocapsid** protein. Rice W G; Turpin J A; Huang M; Clanton D; Buckheit R W Jr; Covell D G; Wallqvist A; McDonnell N B; DeGuzman R N; Summers M F; Zalkow L; Bader J P; Haugwitz R D; Sausville E A. (Laboratory of Antiviral Drug Mechanisms, National Cancer Institute-Frederick Cancer Research and Development Center, SAIC Frederick, Maryland 21702, USA.) Nature medicine, (1997 Mar) 3 (3) 341-5. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB **Nucleocapsid** p7 (NCp7) proteins of **human immunodeficiency virus** type 1 (**HIV**-1) contain two zinc binding domains of the sequence Cys-(X)2-Cys-(X)4-His-(X)4-Cys (CCHC). The spacing pattern and metal-chelating residues (3 Cys, 1 His) of these nucleocapside CCHC zinc fingers are highly conserved among retroviruses. These CCHC domains are required during both the early and late phases of retroviral replication, making them attractive targets for **antiviral** agents. toward that end, we have identified a number of **antiviral** chemotypes that electrophilically attack the sulfur atoms of the zinc-coordinating cysteine residues of the domains. Such nucleocapside **inhibitors** were directly virucidal by preventing the initiation of reverse transcription and blocked formation of infectious virus from cells through modification of CCHC domains within **Gag** precursors. Herein we report that azodicarbonamide (ADA) represents a new compound that inhibits **HIV**-1 and a broad range of retroviruses by targeting the the **nucleocapsid** CCHC domains. Vandevelde et al. also recently disclosed that ADA inhibits **HIV**-1 infection via an unidentified mechanism and that ADA was introduced into Phase I/II clinical trials in Europe for advanced AIDS. These studies distinguish ADA as the first known **nucleocapsid inhibitor** to progress to human trials and provide a lead compound for drug optimization.

=> d hs
'HS' IS NOT A VALID FORMAT FOR FILE 'MEDLINE'

The following are valid formats:

The default display format is BIB.

ABS ---- AB
ALL ---- AN, DN, TI, CM, AU, CS, NC, SO, CY, DT, LA, FS, OS, EM, ED, AB, ST, CT, NA, RN, CN, GEN
BIB ---- AN, DN, TI, CM, AU, CS, NC, SO, CY, DT, LA, FS, OS, EM, ED
CBIB --- AN, DN, TI, CM, AU, CS, NC, SO, CY, DT, LA, FS, OS, EM, ED
DALL --- ALL, delimited for post processing
IABS --- ABS, with a text label
IALL --- ALL, indented with text labels
IBIB --- BIB, indented with text labels
IND ---- ST, CT, NA, RN, CN, GEN
SAM ---- TI, CM, ST, CT, NA, RN, CN, GEN
TRI ---- TI, CM, ST, CT, NA, RN, CN, GEN

HIT ---- All fields containing hit terms
HITIND - IND
KWIC --- All hit terms plus 20 words on either side
OCC ---- List of display fields containing hit terms

Hit terms will be highlighted in all available fields except CM and PY.

To display a particular field or fields, enter the display field codes. For a list of display field codes, enter 'HELP DFIELDS' at an arrow prompt (=>). Examples of formats include: 'BIB'; 'AB'; 'SO,ST'. You may specify the format fields in any order, and the information will be displayed in the same order as the format specification.

The same formats (except for HIT, HITIND, KWIC, and OCC) may be used with the DISPLAY ACC command to display the record for a specified Accession Number.
ENTER DISPLAY FORMAT (BIB):d his
'D' IS NOT A VALID FORMAT FOR FILE 'MEDLINE'
'HIS' IS NOT A VALID FORMAT FOR FILE 'MEDLINE'

The following are valid formats:

The default display format is BIB.

ABS ---- AB
ALL ---- AN, DN, TI, CM, AU, CS, NC, SO, CY, DT, LA, FS, OS, EM,
ED, AB, ST, CT, NA, RN, CN, GEN
BIB ---- AN, DN, TI, CM, AU, CS, NC, SO, CY, DT, LA, FS, OS, EM, ED
CBIB --- AN, DN, TI, CM, AU, CS, NC, SO, CY, DT, LA, FS, OS, EM, ED
DALL --- ALL, delimited for post processing
IABS --- ABS, with a text label
IALL --- ALL, indented with text labels
IBIB --- BIB, indented with text labels
IND ---- ST, CT, NA, RN, CN, GEN
SAM ---- TI, CM, ST, CT, NA, RN, CN, GEN
TRI ---- TI, CM, ST, CT, NA, RN, CN, GEN
TRIAL -- TI, CM, ST, CT, NA, RN, CN, GEN
HIT ---- All fields containing hit terms
HITIND - IND
KWIC --- All hit terms plus 20 words on either side
OCC ---- List of display fields containing hit terms

Hit terms will be highlighted in all available fields except CM and PY.

To display a particular field or fields, enter the display field codes. For a list of display field codes, enter 'HELP DFIELDS' at an arrow prompt (=>). Examples of formats include: 'BIB'; 'AB'; 'SO,ST'. You may specify the format fields in any order, and the information will be displayed in the same order as the format specification.

The same formats (except for HIT, HITIND, KWIC, and OCC) may be used with the DISPLAY ACC command to display the record for a specified Accession Number.
ENTER DISPLAY FORMAT (BIB):ti

L34 ANSWER 1 OF 3 MEDLINE on STN
TI Inhibition of the early phase of **HIV** replication by an isothiazolone, PD 161374.

=> d his

```
      (FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

      FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
                E PREVELIGE P/AU

      FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1              1 S US5716613/PN
L2              1 S US5789245/PN

      FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
                E PREVELIGE P E/AU
L3             35 S E3 OR E4 OR E5
                E KLISHKO V Y/AU
L4              4 S E2-E4
                E GROSS I/AU
                E HOHENBERG H/AU
L5             39 S E3 OR E4
                E CAMPBELL S/AU
L6            864 S E3
                E REIN A/AU
L7             97 S E3
L8              4 S L6 AND L7
                E EHRLICH L S/AU
L9             13 S E3
                E KLIKOVA M/AU
L10             2 S E3
                E SAKALIAN M/AU
L11            14 S E3 OR E4
                E SMITH A J/AU
L12           594 S E3
L13            17 S L12 AND PY=1990
L14             1 S L13 AND ASSEMBLED
                E LEIS J/AU
L15            65 S E3
L16             8 S L15 AND PY=1988

     FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
L17         33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L18         23040 S L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOC
L19            66 S L18 AND (IN VITRO ASSEMBLY)
L20            66 S L19 AND (ASSEMBLY OR MORPHOGENESIS OR FORMATION OR VIRUS-LIKE
L21            35 S L20 AND AY<2001
L22          2371 S L17 AND (GAG/CLM OR CA/CLM OR MA/CLM OR NC/CLM OR CAPSID/CLM
L23           664 S L22 AND (ASSEMBLY/CLM OR PARTICLE?/CLM OR MORPHOGENESIS/CLM O
L24           248 S L23 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L25           115 S L24 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 20:39:11 ON 24 JUN 2004
                E TRONO D/AU
L26           106 S E3 OR E4
L27             1 S L26 AND (TRANS-DOMINANT)
L28            11 S L26 AND (GAG)
L29        142537 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L30          6071 S L29 AND (GAG OR MA OR CA OR NC OR MATRIX OR CAPSID OR NUCLEOC
L31            12 S L30 AND (IN VITRO ASSEMBLY)
L32          1088 S L30 AND (INHIBITOR? OR ANTIVIRAL?)
L33             6 S L32 AND (SMALL MOLECULE)
L34             3 S L32 AND (GAG INHIBITOR? OR CAPSID INHIBITOR? OR MATRIX INHIBI

=> s l32 and py=1999
        458927 PY=1999
L35            88 L32 AND PY=1999

=> s l35 and (gag/ti or matrix/ti or capsid/ti or nucleocapsid/ti)
           1667 GAG/TI
```

2038 CAPSID/TI
1033 NUCLEOCAPSID/TI
L36 15 L35 AND (GAG/TI OR MATRIX/TI OR CAPSID/TI OR NUCLEOCAPSID/TI)

=> d l36, ti,1-5

L36 ANSWER 1 OF 15 MEDLINE on STN
TI **Human immunodeficiency virus**-1-tat induces **matrix** metalloproteinase-9 in monocytes through protein tyrosine phosphatase-mediated activation of nuclear transcription factor NF-kappaB.

L36 ANSWER 2 OF 15 MEDLINE on STN
TI Multiple effects of an anti-**human immunodeficiency virus nucleocapsid inhibitor** on virus morphology and replication.

L36 ANSWER 3 OF 15 MEDLINE on STN
TI A conserved dileucine-containing motif in p6(**gag**) governs the particle association of Vpx and Vpr of simian immunodeficiency viruses SIV(mac) and SIV(agm).

L36 ANSWER 4 OF 15 MEDLINE on STN
TI Involvement of **matrix** metalloproteinases in **human immunodeficiency virus** type 1-induced replication by clinical Mycobacterium avium isolates.

L36 ANSWER 5 OF 15 MEDLINE on STN
TI Cloning and characterization of hIF2, a human homologue of bacterial translation initiation factor 2, and its interaction with **HIV**-1 **matrix**.

=> d l36,ti,6-15

L36 ANSWER 6 OF 15 MEDLINE on STN
TI Competitive inhibition of **human immunodeficiency virus** type-1 protease by the **Gag**-Pol transframe protein.

L36 ANSWER 7 OF 15 MEDLINE on STN
TI Coupled integration of **human immunodeficiency virus** type 1 cDNA ends by purified integrase in vitro: stimulation by the viral **nucleocapsid** protein.

L36 ANSWER 8 OF 15 MEDLINE on STN
TI A mimic of **HIV**-1 **nucleocapsid** protein impairs reverse transcription and displays **antiviral** activity.

L36 ANSWER 9 OF 15 MEDLINE on STN
TI **Gag** protein epitopes recognized by CD4(+) T-helper lymphocytes from equine infectious anemia virus-infected carrier horses.

L36 ANSWER 10 OF 15 MEDLINE on STN
TI Evidence of interactions between the **nucleocapsid** protein NCp7 and the reverse transcriptase of **HIV**-1.

L36 ANSWER 11 OF 15 MEDLINE on STN
TI Nucleomimetic strategy for the inhibition of **HIV**-1 **nucleocapsid** protein NCp7 activities.

L36 ANSWER 12 OF 15 MEDLINE on STN
TI Maturation-induced conformational changes of **HIV**-1 **capsid** protein and identification of two high affinity sites for cyclophilins in the C-terminal domain.

L36 ANSWER 13 OF 15 MEDLINE on STN
TI Acute effects of pathogenic simian-**human immunodeficiency virus** challenge on vaccine-induced cellular and humoral immune responses to

Gag in Rhesus Macaques.

L36 ANSWER 14 OF 15 MEDLINE on STN
TI Synthesis and biological properties of novel pyridinioalkanoyl thiolesters (PATE) as anti-**HIV**-1 agents that target the viral **nucleocapsid** protein zinc fingers.

L36 ANSWER 15 OF 15 MEDLINE on STN
TI A human cell line constitutively expressing **HIV**-1 **Gag** and **Gag**-Pol gene products.

=> d l36,cbib,ab,8

L36 ANSWER 8 OF 15 MEDLINE on STN
1999238452. PubMed ID: 10220388. A mimic of **HIV**-1 **nucleocapsid** protein impairs reverse transcription and displays **antiviral** activity. Druillennec S; Dong C Z; Escaich S; Gresh N; Bousseau A; Roques B P; Fournie-Zaluski M C. (Departement de Pharmacochimie Moleculaire et Structurale, U 266 Institut National de la Sante et de la Recherche Medicale, Unite Mixte de Recherche, France.) Proceedings of the National Academy of Sciences of the United States of America, **(1999 Apr 27)** 96 (9) 4886-91. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Combined inhibition of **HIV**-1 reverse transcriptase and protease has significantly improved the treatment of AIDS. Nevertheless, resistance to these drugs occurs rapidly because of viral mutations, emphasizing the importance of identifying novel retroviral targets to develop new drug combinations. The critical role played by the **nucleocapsid** protein NCp7 of **HIV**-1 at different steps of the retrovirus life cycle makes it an attractive target for the development of new **antiviral** agents. NCp7 contains two highly conserved zinc fingers and is characterized by a three-dimensional structure that cannot be modified without a complete loss of infectivity of mutated viruses. Based on these structural data, we report that RB 2121, a cyclic peptide designed to mimic several essential biological determinants of NCp7, displays **antiviral** activity by inhibiting **HIV**-1 replication in CEM-4 cells infected by **HIV**-1. In vitro, RB 2121 does not interfere with **HIV**-1 cell entry and viral enzymes but is able to inhibit the annealing activities of NCp7 by recognizing nucleic acids. Analysis of proviral DNA synthesis by means of PCR has shown that RB 2121 acts at an early step of the retrovirus life cycle by inducing a dose-dependent reduction in transcribed DNA levels through inhibition of NCp7-reverse transcriptase interaction. Because of its original mechanism of action, RB 2121 provides an interesting lead for the rational development of new anti-**HIV**-1 agents that could be associated advantageously with enzyme **inhibitors** to counteract rapid virus mutations and resistance problems observed in tritherapies.

=> d l36,cbib,ab,11

L36 ANSWER 11 OF 15 MEDLINE on STN
1999196314. PubMed ID: 10098678. Nucleomimetic strategy for the inhibition of **HIV**-1 **nucleocapsid** protein NCp7 activities. Druillennec S; Meudal H; Roques B P; Fournie-Zaluski M C. (Departement de Pharmacochimie Moleculaire et Structurale INSERM U266-CNRS UMR 8600 UFR des Sciences Pharmaceutiques et Biologiques, Paris, France.) Bioorganic & medicinal chemistry letters, **(1999 Feb 22)** 9 (4) 627-32. Journal code: 9107377. ISSN: 0960-894X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB We report the synthesis and biological properties of three modified dinucleotides T*G, G*T and T*T in which the natural phosphodiester linkage has been replaced by a methylene carboxamide unit. They have been designed to act as nucleomimetics of a sequence recognized by the **HIV**-1 **nucleocapsid** protein NCp7 and to inhibit this interaction.

L36 ANSWER 6 OF 15 MEDLINE on STN
1999348275. PubMed ID: 10419458. Competitive inhibition of **human immunodeficiency virus** type-1 protease by the **Gag**-Pol transframe protein. Paulus C; Hellebrand S; Tessmer U; Wolf H; Krausslich H G; Wagner R. (Institut fur Medizinische Mikrobiologie und Hygiene, Universitat Regensburg, Franz-Josef-Strauss-Allee 11, D-93053 Regensburg, Germany.) Journal of biological chemistry, **(1999 Jul 30)** 274 (31) 21539-43. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus** type-1 (**HIV**-1) transframe protein p6* is located between the structural and enzymatic domains of the **Gag**-Pol polyprotein, flanked by the **nucleocapsid** (**NC**) and the protease (PR) domain at its amino and carboxyl termini, respectively. Here, we report that recombinant highly purified **HIV**-1 p6* specifically inhibits mature **HIV**-1 PR activity. Kinetic analyses and cross-linking experiments revealed a competitive mechanism for PR inhibition by p6*. We further demonstrate that the four carboxyl-terminal residues of p6* are essential but not sufficient for p6*-mediated inhibition of PR activity. Based on these results, we suggest a role of the transframe protein p6* in regulating **HIV**-1 PR activity during viral replication.

=> d his

(FILE 'HOME' ENTERED AT 19:26:55 ON 24 JUN 2004)

FILE 'MEDLINE' ENTERED AT 19:27:07 ON 24 JUN 2004
E PREVELIGE P/AU

FILE 'USPATFULL' ENTERED AT 19:28:11 ON 24 JUN 2004
L1 1 S US5716613/PN
L2 1 S US5789245/PN

FILE 'MEDLINE' ENTERED AT 19:40:03 ON 24 JUN 2004
E PREVELIGE P E/AU
L3 35 S E3 OR E4 OR E5
E KLISHKO V Y/AU
L4 4 S E2-E4
E GROSS I/AU
E HOHENBERG H/AU
L5 39 S E3 OR E4
E CAMPBELL S/AU
L6 864 S E3
E REIN A/AU
L7 97 S E3
L8 4 S L6 AND L7
E EHRLICH L S/AU
L9 13 S E3
E KLIKOVA M/AU
L10 2 S E3
E SAKALIAN M/AU
L11 14 S E3 OR E4
E SMITH A J/AU
L12 594 S E3
L13 17 S L12 AND PY=1990
L14 1 S L13 AND ASSEMBLED
E LEIS J/AU
L15 65 S E3
L16 8 S L15 AND PY=1988

FILE 'USPATFULL' ENTERED AT 20:27:08 ON 24 JUN 2004
L17 33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L18 23040 S L17 AND (GAG OR CA OR NC OR MA OR CAPSID OR MATRIX OR NUCLEOC
L19 66 S L18 AND (IN VITRO ASSEMBLY)

```
L21          35 S L20 AND AY<2001
L22        2371 S L17 AND (GAG/CLM OR CA/CLM OR MA/CLM OR NC/CLM OR CAPSID/CLM
L23         664 S L22 AND (ASSEMBLY/CLM OR PARTICLE?/CLM OR MORPHOGENESIS/CLM O
L24         248 S L23 AND (HIV/CLM OR HUMAN IMMUNODEFICIENCY VIRUS/CLM)
L25         115 S L24 AND AY<2001

     FILE 'MEDLINE' ENTERED AT 20:39:11 ON 24 JUN 2004
                E TRONO D/AU
L26         106 S E3 OR E4
L27           1 S L26 AND (TRANS-DOMINANT)
L28          11 S L26 AND (GAG)
L29      142537 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L30        6071 S L29 AND (GAG OR MA OR CA OR NC OR MATRIX OR CAPSID OR NUCLEOC
L31          12 S L30 AND (IN VITRO ASSEMBLY)
L32        1088 S L30 AND (INHIBITOR? OR ANTIVIRAL?)
L33           6 S L32 AND (SMALL MOLECULE)
L34           3 S L32 AND (GAG INHIBITOR? OR CAPSID INHIBITOR? OR MATRIX INHIBI
L35          88 S L32 AND PY=1999
L36          15 S L35 AND (GAG/TI OR MATRIX/TI OR CAPSID/TI OR NUCLEOCAPSID/TI)

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 21:02:30 ON 24 JUN 2004
```